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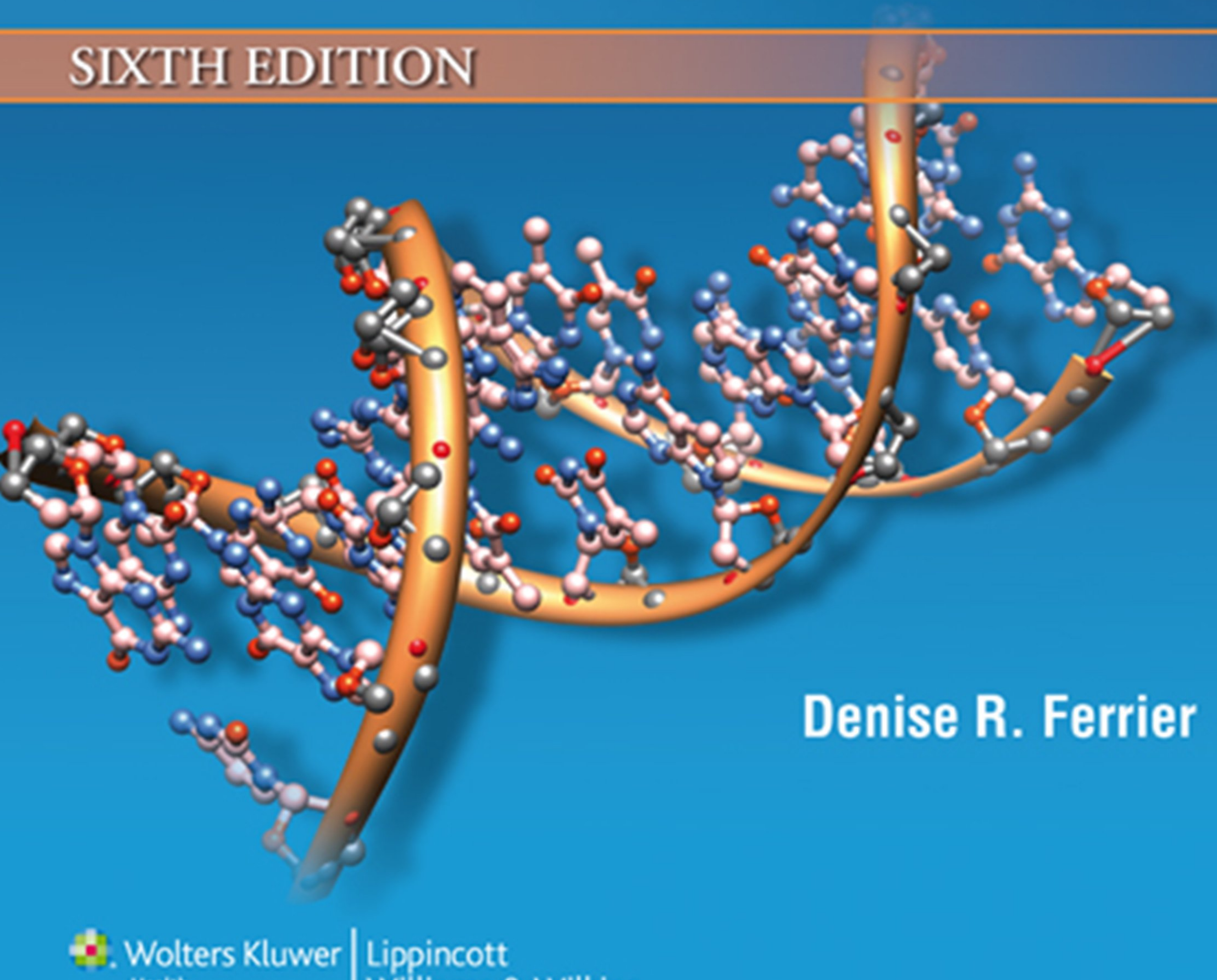
Lippincott's Illustrated Reviews

Biochemistry

SIXTH EDITION



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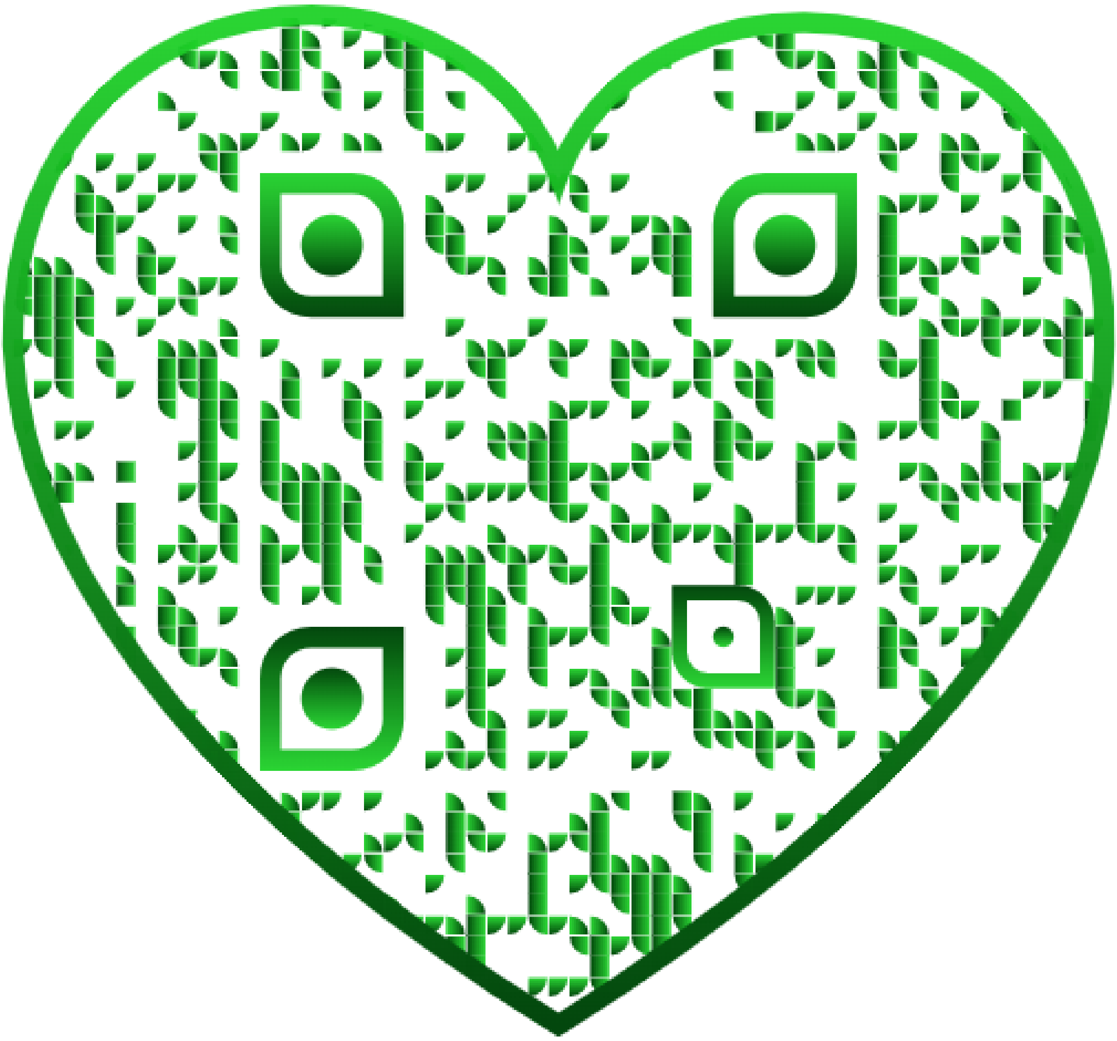

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UNIT I:
Protein Structure and Function

Amino Acids

1

I. OVERVIEW

Proteins are the most abundant and functionally diverse molecules in living systems. Virtually every life process depends on this class of macromolecules. For example, enzymes and polypeptide hormones direct and regulate metabolism in the body, whereas contractile proteins in muscle permit movement. In bone, the protein collagen forms a framework for the deposition of calcium phosphate crystals, acting like the steel cables in reinforced concrete. In the bloodstream, proteins, such as hemoglobin and plasma albumin, shuttle molecules essential to life, whereas immunoglobulins fight infectious bacteria and viruses. In short, proteins display an incredible diversity of functions, yet all share the common structural feature of being linear polymers of amino acids. This chapter describes the properties of amino acids. Chapter 2 explores how these simple building blocks are joined to form proteins that have unique three-dimensional structures, making them capable of performing specific biologic functions.

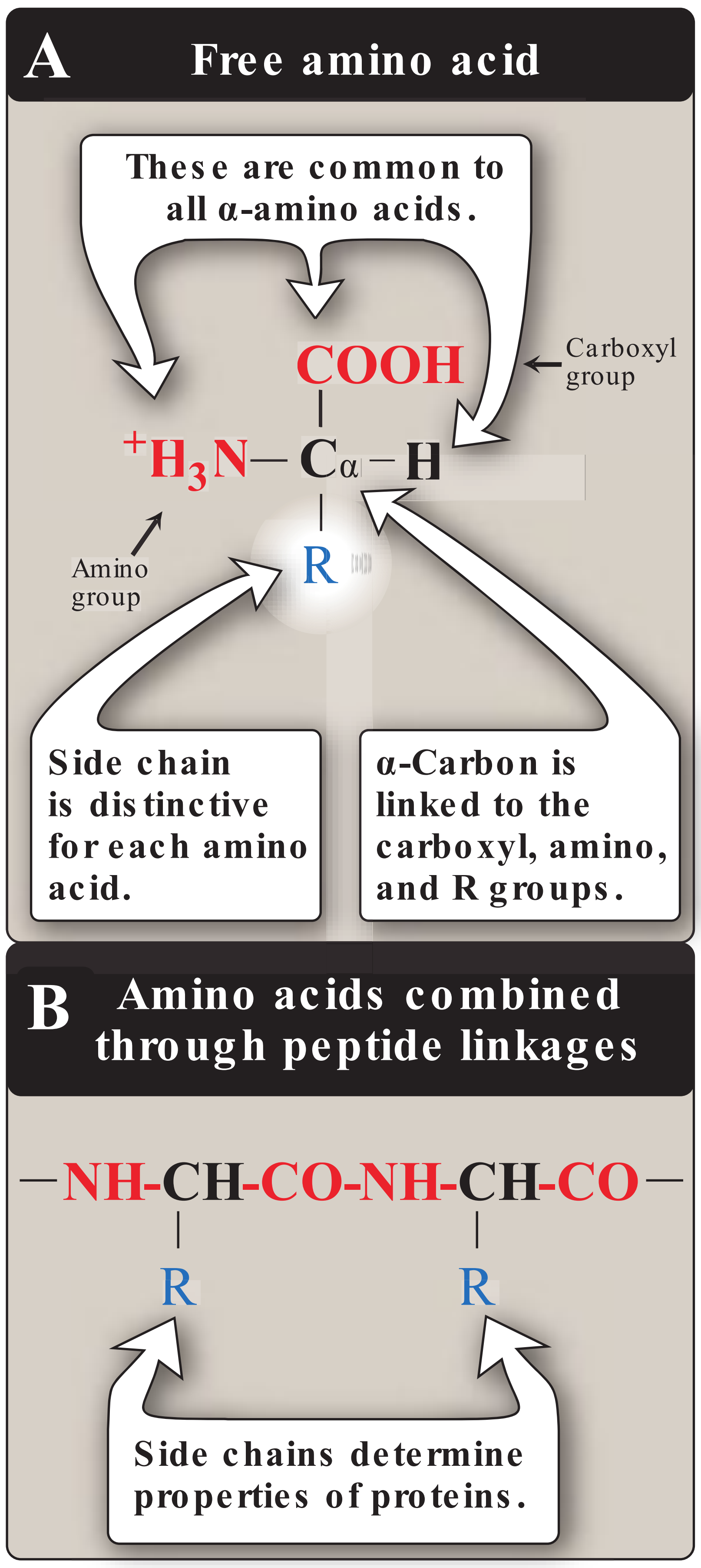

A
Free amino acid
These are common to all α-amino acids.
COOH
Carboxyl group
$^+H_3N-C\alpha-H$
Amino group
R
Side chain is distinctive for each amino acid.
α-Carbon is linked to the carboxyl, amino, and R groups.
B
Amino acids combined through peptide linkages
—NH-CH-CO-NH-CH-CO—
R
R
Side chains determine properties of proteins.

Figure 1.1
Structural features of amino acids (shown in their fully protonated form).

II. STRUCTURE

Although more than 300 different amino acids have been described in nature, only 20 are commonly found as constituents of mammalian proteins. [Note: These are the only amino acids that are coded for by DNA, the genetic material in the cell (see p. 395).] Each amino acid has a carboxyl group, a primary amino group (except for proline, which has a secondary amino group), and a distinctive side chain ("R group") bonded to the a-carbon atom (Figure 1.1A). At physiologic pH (approximately 7.4), the carboxyl group is dissociated, forming the negatively charged carboxylate ion ($-COO^-$), and the amino group is protonated ($-NH_3^+$). In proteins, almost all of these carboxyl and amino groups are combined through peptide linkage and, in general, are not available for chemical reaction except for hydrogen bond formation (Figure 1.1B). Thus, it is the nature of the side chains that ultimately dictates the role an amino acid plays in a protein. It is, therefore, useful to classify the amino acids according to the properties of their side chains, that is, whether they are nonpolar (have an even distribution of electrons) or polar (have an uneven distribution of electrons, such as acids and bases) as shown in Figures 1.2 and 1.3.

A. Amino acids with nonpolar side chains

Each of these amino acids has a nonpolar side chain that does not gain or lose protons or participate in hydrogen or ionic bonds (see Figure 1.2). The side chains of these amino acids can be thought of as "oily" or lipid-like, a property that promotes hydrophobic interactions (see Figure 2.10, p. 19).

1. **Location of nonpolar amino acids in proteins:** In proteins found in aqueous solutions (a polar environment) the side chains of the nonpolar amino acids tend to cluster together in the interior of the protein (Figure 1.4). This phenomenon, known as the hydrophobic

NONPOLAR SIDE CHAINS

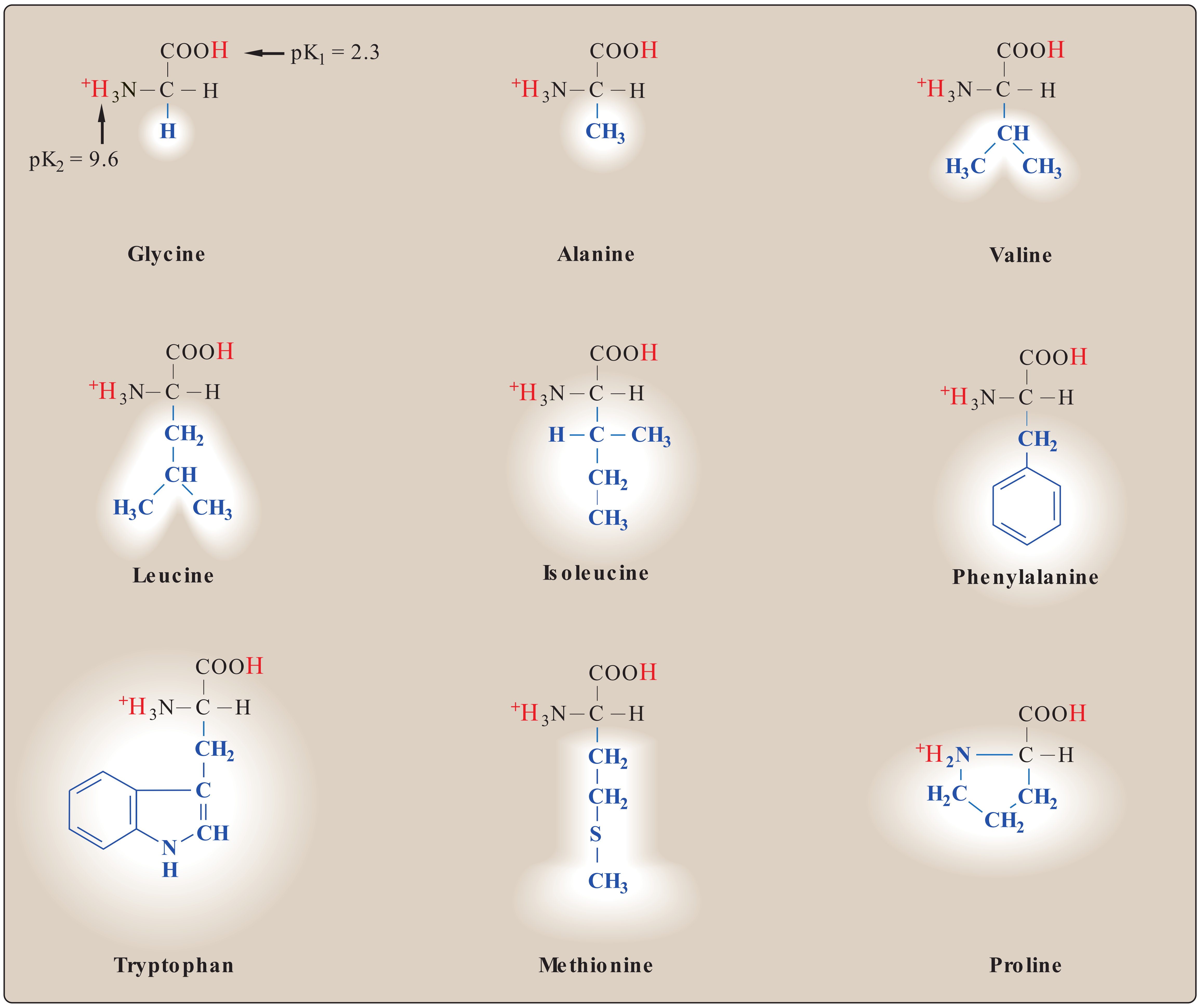
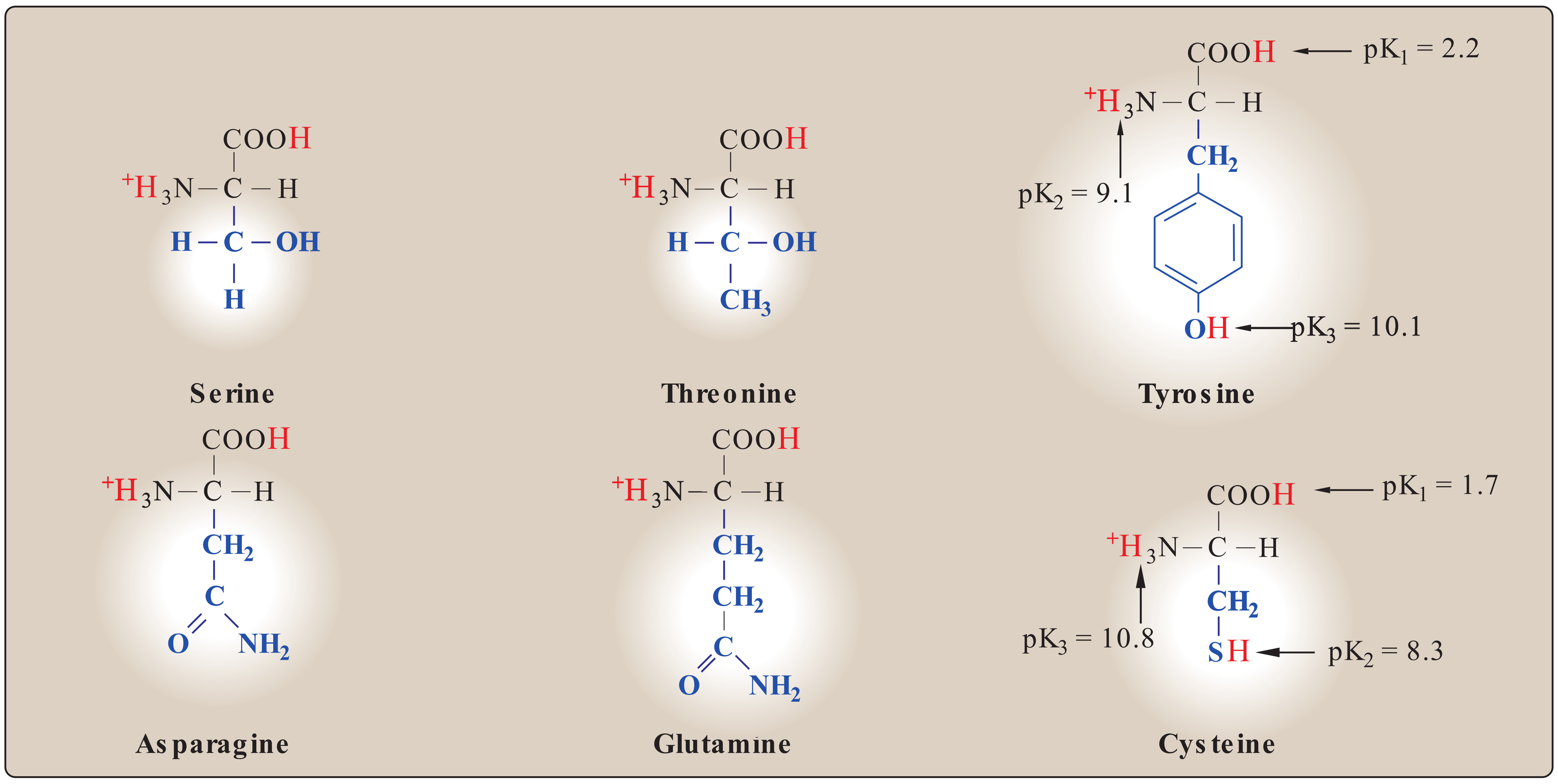
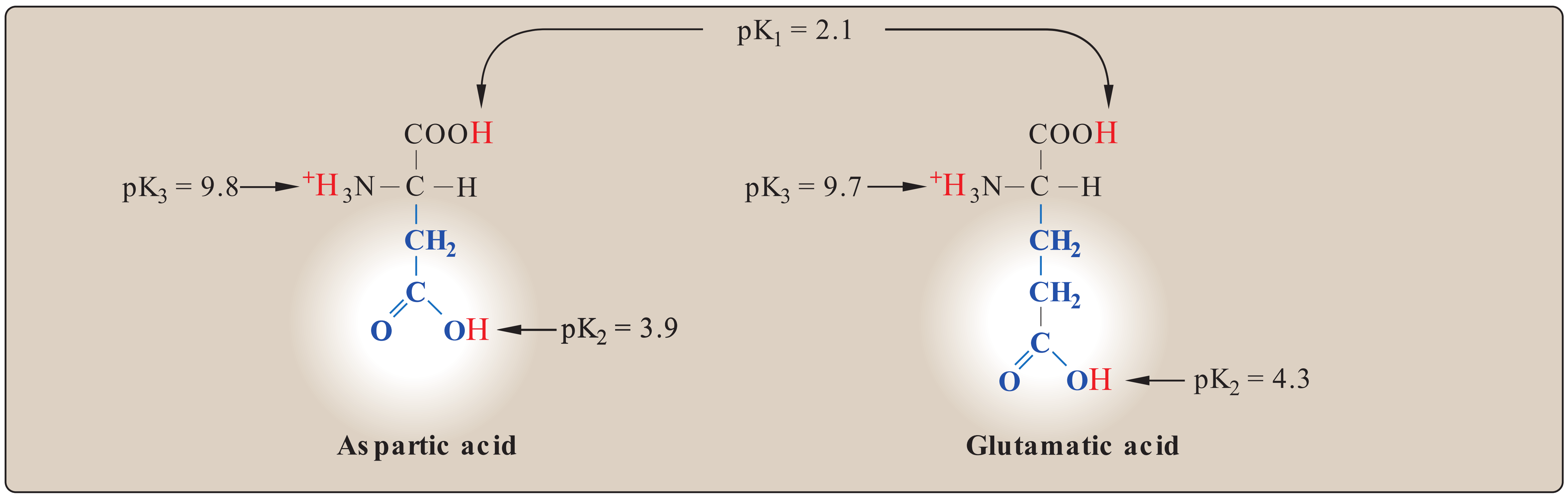


Figure 1.2
Classification of the 20 amino acids commonly found in proteins, according to the charge and polarity of their side chains at acidic pH is shown here and continues in Figure 1.3. Each amino acid is shown in its fully protonated form, with dissociable hydrogen ions represented in red print. The pK values for the α-carboxyl and α-amino groups of the nonpolar amino acids are similar to those shown for glycine.

UNCHARGED POLAR SIDE CHAINS


Serine
Threonine
pK$_1$ = 2.2
pK$_2$ = 9.1
pK$_3$ = 10.1
Tyrosine
Asparagine
Glutamine
pK$_1$ = 1.7
pK$_3$ = 10.8
pK$_2$ = 8.3
Cysteine

ACIDIC SIDE CHAINS


pK$_1$ = 2.1
pK$_3$ = 9.8
pK$_2$ = 3.9
pK$_3$ = 9.7
pK$_2$ = 4.3
Aspartic acid
Glutamatic acid

BASIC SIDE CHAINS

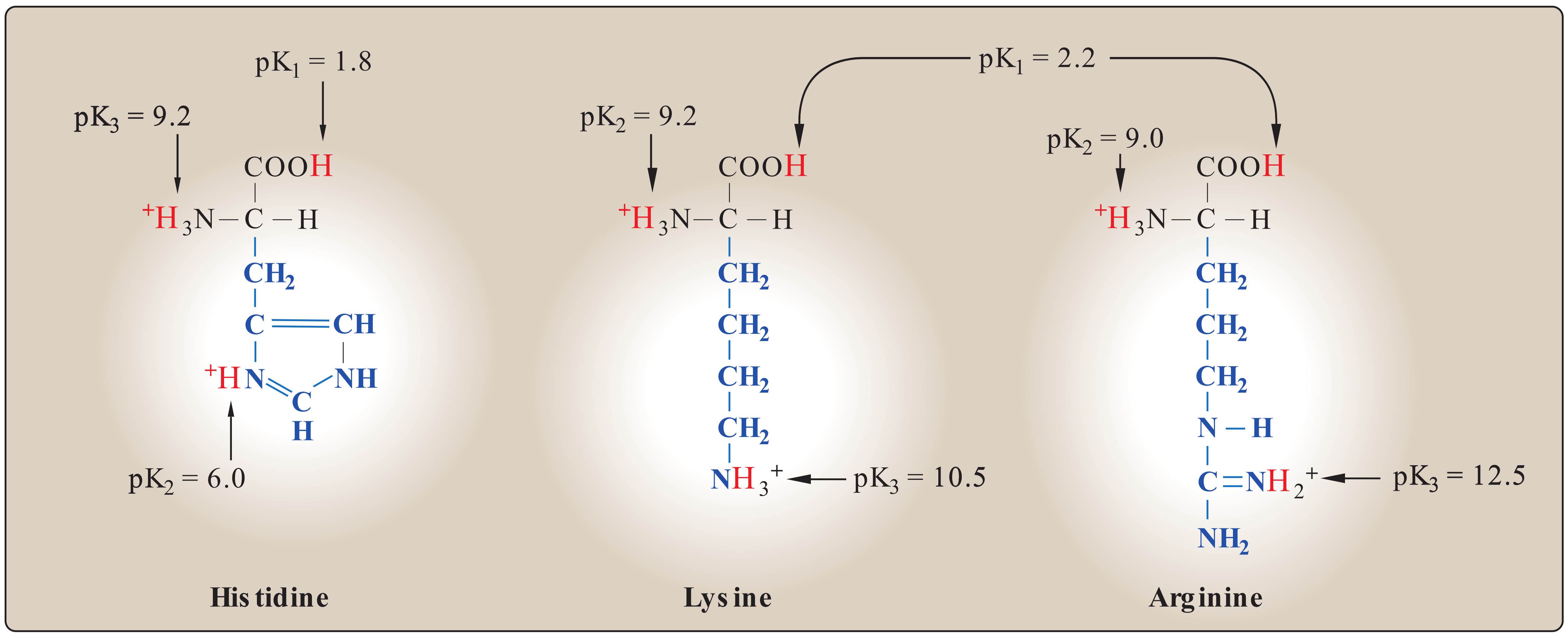

pK$_1$ = 1.8
pK$_3$ = 9.2
pK$_2$ = 6.0
Histidine
pK$_1$ = 2.2
pK$_2$ = 9.2
pK$_3$ = 10.5
Lysine
pK$_2$ = 9.0
pK$_3$ = 12.5
Arginine

Figure 1.3
Classification of the 20 amino acids commonly found in proteins, according to the charge and polarity of their side chains at acidic pH (continued from Figure 1.2).

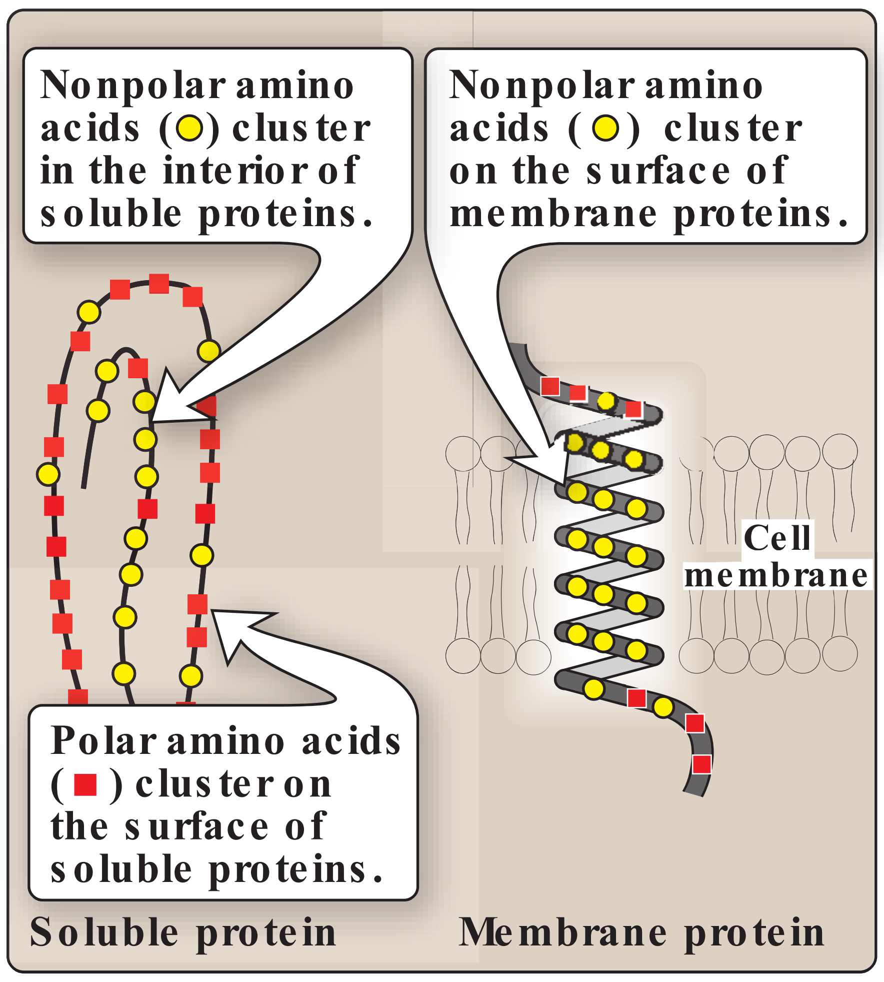

Nonpolar amino acids (●) cluster in the interior of soluble proteins.
Nonpolar amino acids (●) cluster on the surface of membrane proteins.
Cell membrane
Polar amino acids (■) cluster on the surface of soluble proteins.
Soluble protein
Membrane protein

Figure 1.4
Location of nonpolar amino acids in soluble and membrane proteins.

effect, is the result of the hydrophobicity of the nonpolar R groups, which act much like droplets of oil that coalesce in an aqueous environment. The nonpolar R groups, thus, fill up the interior of the folded protein and help give it its three-dimensional shape. However, for proteins that are located in a hydrophobic environment, such as a membrane, the nonpolar R groups are found on the outside surface of the protein, interacting with the lipid environment see Figure 1.4. The importance of these hydrophobic interactions in stabilizing protein structure is discussed on p. 19.

> Sickle cell anemia, a sickling disease of red blood cells, results from the replacement of polar glutamate with nonpolar valine at the sixth position in the b subunit of hemoglobin (see p. 36).

2. **Proline:** Proline differs from other amino acids in that its side chain and a-amino N form a rigid, five-membered ring structure (Figure 1.5). Proline, then, has a secondary (rather than a primary) amino group. It is frequently referred to as an "imino acid." The unique geometry of proline contributes to the formation of the fibrous structure of collagen (see p. 45) and often interrupts the a-helices found in globular proteins (see p. 26).

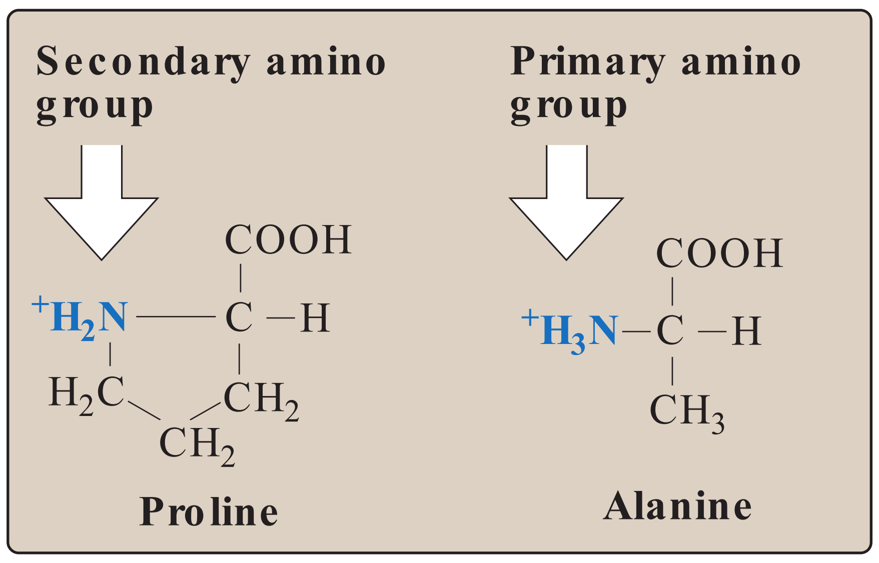

Secondary amino group
COOH
^+H_2N
C—H
H_2C
CH_2
CH_2
Proline
Primary amino group
COOH
^+H_3N—C—H
CH_3
Alanine

Figure 1.5
Comparison of the secondary amino group found in proline with the primary amino group found in other amino acids such as alanine.

B. Amino acids with uncharged polar side chains

These amino acids have zero net charge at physiologic pH, although the side chains of cysteine and tyrosine can lose a proton at an alkaline pH (see Figure 1.3). Serine, threonine, and tyrosine each contain a polar hydroxyl group that can participate in hydrogen bond formation (Figure 1.6). The side chains of asparagine and glutamine each contain a carbonyl group and an amide group, both of which can also participate in hydrogen bonds.

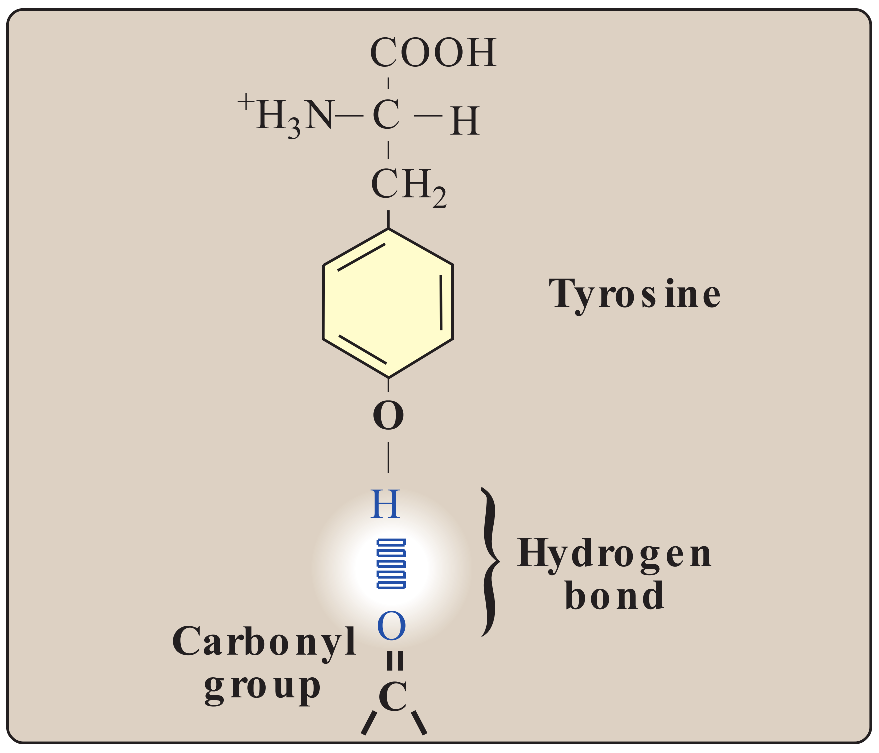

COOH
^+H_3N–C–H
CH_2
Tyrosine
O
H
Hydrogen bond
Carbonyl group
O
C

Figure 1.6
Hydrogen bond between the phenolic hydroxyl group of tyrosine and another molecule containing a carbonyl group.

1. **Disulfide bond:** The side chain of cysteine contains a sulfhydryl (thiol) group (–SH), which is an important component of the active site of many enzymes. In proteins, the –SH groups of two cysteines can be oxidized to form a covalent cross-link called a disulfide bond (–S–S–). Two disulfide-linked cysteines are referred to as "cystine." (See p. 19 for a further discussion of disulfide bond formation.)

> Many extracellular proteins are stabilized by disulfide bonds. Albumin, a blood protein that functions as a transporter for a variety of molecules, is an example.

2. **Side chains as sites of attachment for other compounds:** The polar hydroxyl group of serine; threonine; and, rarely, tyrosine, can serve as a site of attachment for structures such as a phosphate group. In addition, the amide group of asparagine, as well as the hydroxyl group of serine or threonine, can serve as a site of attachment for oligosaccharide chains in glycoproteins (see p. 165).

C. Amino acids with acidic side chains

The amino acids aspartic and glutamic acid are proton donors. At physiologic pH, the side chains of these amino acids are fully ionized, containing a negatively charged carboxylate group ($–COO^–$). They are, therefore, called aspartate or glutamate to emphasize that these amino acids are negatively charged at physiologic pH (see Figure 1.3).

D. Amino acids with basic side chains

The side chains of the basic amino acids accept protons (see Figure 1.3). At physiologic pH, the R groups of lysine and arginine are fully ionized and positively charged. In contrast, histidine is weakly basic, and the free amino acid is largely uncharged at physiologic pH. However, when histidine is incorporated into a protein, its R group can be either positively charged (protonated) or neutral, depending on the ionic environment provided by the protein. This is an important property of histidine that contributes to the buffering role it plays in the functioning of proteins such as hemoglobin (see p. 31). [Note: Histidine is the only amino acid with a side chain that can ionize within the physiologic pH range.]

E. Abbreviations and symbols for commonly occurring amino acids

Each amino acid name has an associated three-letter abbreviation and a one-letter symbol (Figure 1.7). The one-letter codes are determined by the following rules.

1. **Unique first letter:** If only one amino acid begins with a given letter, then that letter is used as its symbol. For example, V = valine.

2. **Most commonly occurring amino acids have priority:** If more than one amino acid begins with a particular letter, the most common of these amino acids receives this letter as its symbol. For example, glycine is more common than glutamate, so G = glycine.

3. **Similar sounding names:** Some one-letter symbols sound like the amino acid they represent. For example, F = phenylalanine, or W = tryptophan ("twyptophan" as Elmer Fudd would say).

4. **Letter close to initial letter:** For the remaining amino acids, a one-letter symbol is assigned that is as close in the alphabet as possible to the initial letter of the amino acid, for example, K = lysine. Furthermore, B is assigned to Asx, signifying either aspartic acid or asparagine, Z is assigned to Glx, signifying either glutamic acid or glutamine, and X is assigned to an unidentified amino acid.

1 Unique first letter:

Cysteine = Cys = C
Histidine = His = H
Isoleucine = Ile = I
Methionine = Met = M
Serine = Ser = S
Valine = Val = V

2 Most commonly occurring amino acids have priority:

Alanine = Ala = A
Glycine = Gly = G
Leucine = Leu = L
Proline = Pro = P
Threonine = Thr = T

3 Similar sounding names:

Arginine = Arg = R ("aRginine")
Asparagine = Asn = N (contains N)
Aspartate = Asp = D ("asparDic")
Glutamate = Glu = E ("glutEmate")
Glutamine = Gln = Q ("Q-tamine")
Phenylalanine = Phe = F ("Fenylalanine")
Tyrosine = Tyr = Y ("tYrosine")
Tryptophan = Trp = W (double ring in the molecule)

4 Letter close to initial letter:

Aspartate or asparagine = Asx = B (near A)
Glutamate or glutamine = Glx = Z
Lysine = Lys = K (near L)
Undetermined amino acid = X

Figure 1.7
Abbreviations and symbols for the commonly occurring amino acids.

F. Optical properties of amino acids

The a-carbon of an amino acid is attached to four different chemical groups (asymmetric) and is, therefore, a chiral, or optically active carbon atom. Glycine is the exception because its a-carbon has two hydrogen substituents. Amino acids with an asymmetric center at the a-carbon can exist in two forms, designated D and L, that are mirror images of each other (Figure 1.8). The two forms in each pair are termed stereoisomers, optical isomers, or enantiomers. All amino acids found in proteins are of the L configuration. However, D-amino acids are found in some antibiotics and in bacterial cell walls. (See p. 252 for a discussion of D-amino acids.)

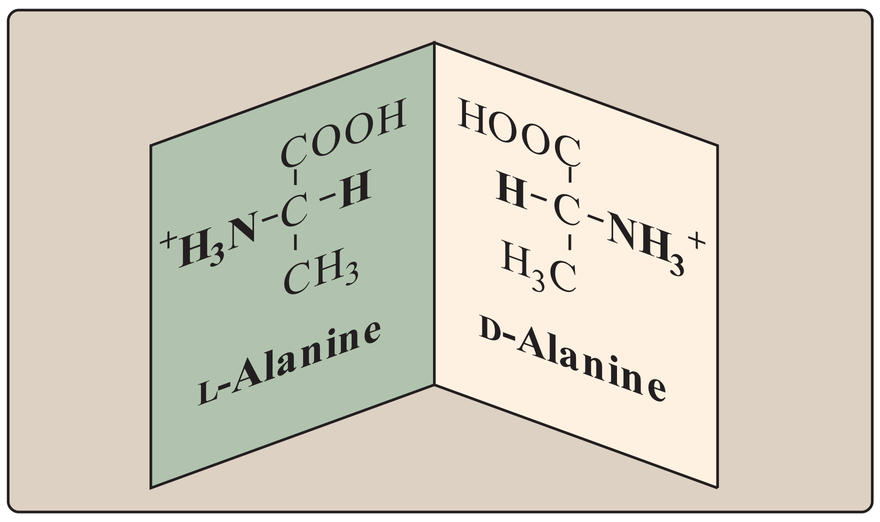


Figure 1.8
D and L forms of alanine are mirror images.

III. ACIDIC AND BASIC PROPERTIES OF AMINO ACIDS

Amino acids in aqueous solution contain weakly acidic a-carboxyl groups and weakly basic a-amino groups. In addition, each of the acidic and basic amino acids contains an ionizable group in its side chain. Thus, both free amino acids and some amino acids combined in peptide linkages can act as buffers. Recall that acids may be defined as proton donors and bases as proton acceptors. Acids (or bases) described as "weak" ionize to only a limited extent. The concentration of protons in aqueous solution is expressed as pH, where pH = log 1/[H^+] or – log [H^+]. The quantitative relationship between the pH of the solution and concentration of a weak acid (HA) and its conjugate base (A^-) is described by the Henderson-Hasselbalch equation.

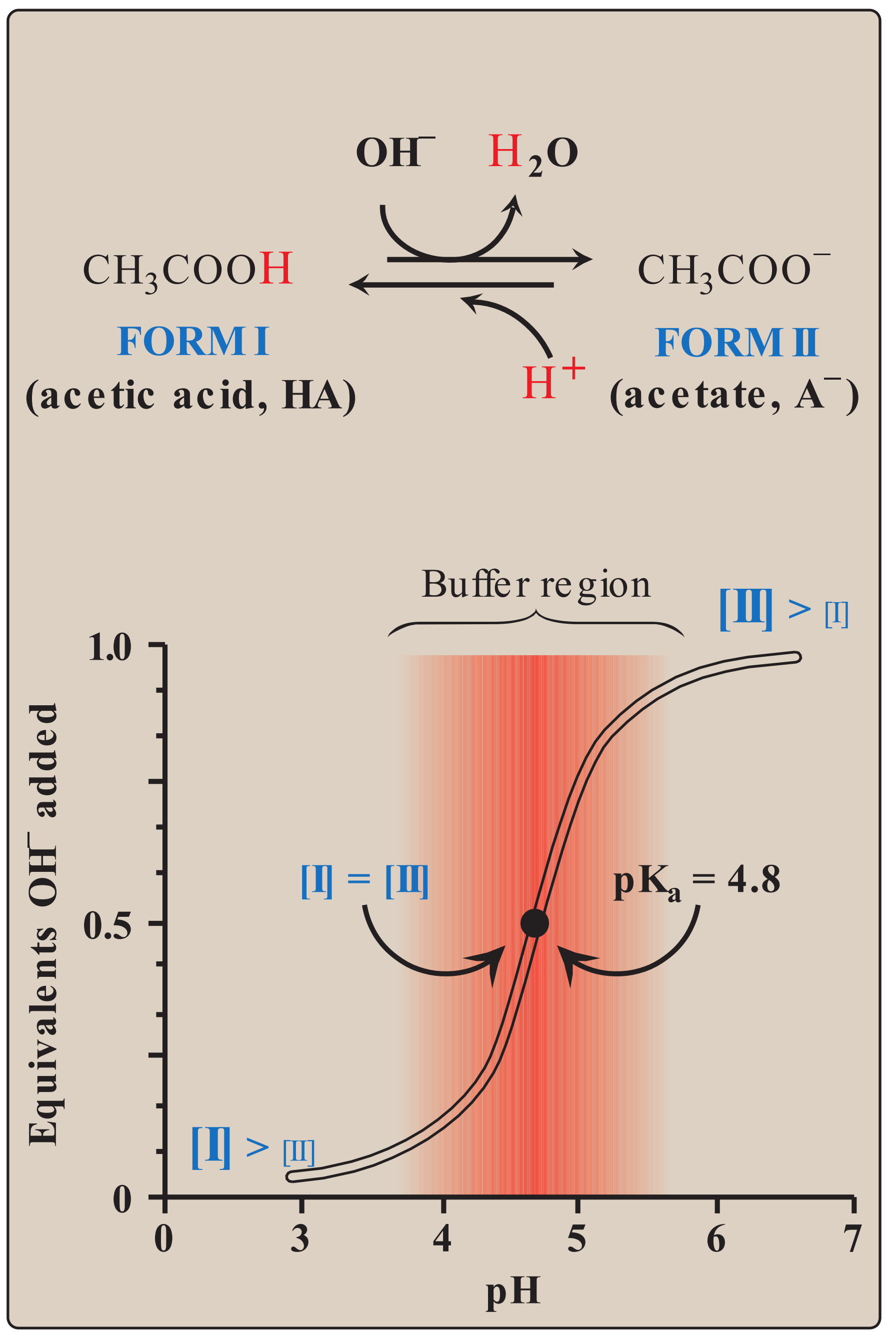


Figure 1.9
Titration curve of acetic acid.

A. Derivation of the equation

Consider the release of a proton by a weak acid represented by HA:

HA	$\leftrightarrows$	H^+	+	A^-
weak acid		proton		salt form or conjugate base

The "salt" or conjugate base, A^-, is the ionized form of a weak acid. By definition, the dissociation constant of the acid, K_a, is

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

[Note: The larger the K_a, the stronger the acid, because most of the HA has dissociated into H^+ and A^-. Conversely, the smaller the K_a, the less acid has dissociated and, therefore, the weaker the acid.] By solving for the [H^+] in the above equation, taking the logarithm of both sides of the equation, multiplying both sides of the equation by –1, and substituting pH = – log [H^+] and $pK_a = -\log K_a$, we obtain the Henderson-Hasselbalch equation:

$$\boxed{pH = pK_a + \log \frac{[A^-]}{[HA]}}$$

B. Buffers

A buffer is a solution that resists change in pH following the addition of an acid or base. A buffer can be created by mixing a weak acid (HA) with its conjugate base (A^-). If an acid such as HCl is added to a buffer, A^- can neutralize it, being converted to HA in the process. If a base is added, HA can neutralize it, being converted to A^- in the process. Maximum buffering capacity occurs at a pH equal to the pK_a, but a conjugate acid–base pair can still serve as an effective buffer when the pH of a solution is within approximately ±1 pH unit of the pK_a. If the amounts of HA and A^- are equal, the pH is equal to the pK_a. As shown in Figure

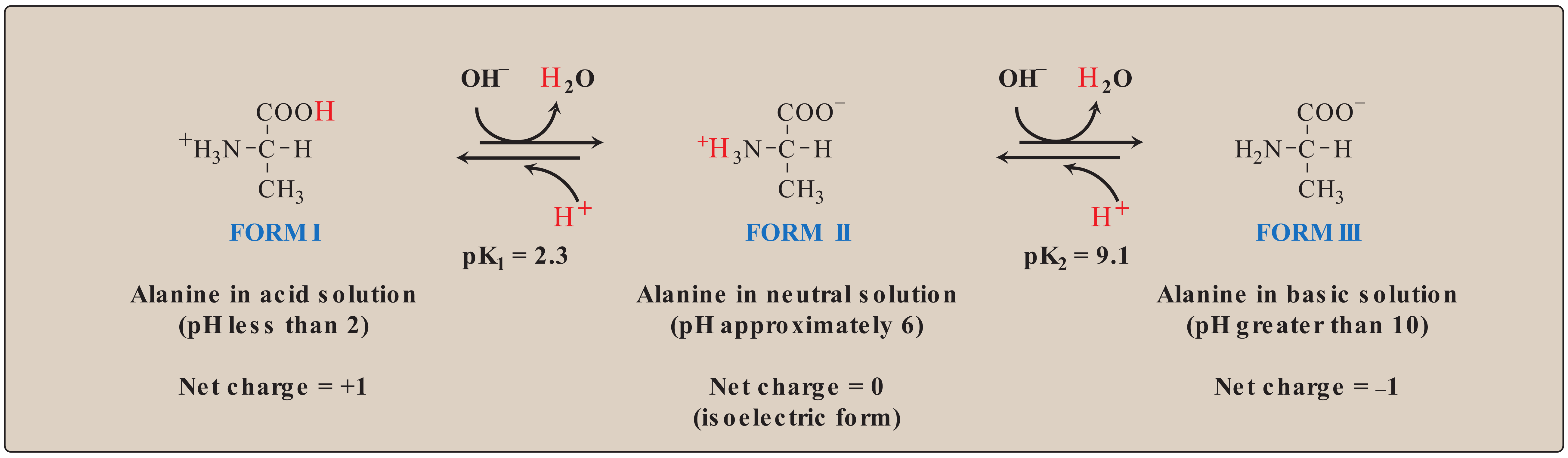

OH⁻
H₂O
OH⁻
H₂O
COOH
⁺H₃N-C-H
CH₃
COO⁻
⁺H₃N-C-H
CH₃
COO⁻
H₂N-C-H
CH₃
H⁺
H⁺
FORM I
pK₁ = 2.3
FORM II
pK₂ = 9.1
FORM III
Alanine in acid solution
(pH less than 2)
Alanine in neutral solution
(pH approximately 6)
Alanine in basic solution
(pH greater than 10)
Net charge = +1
Net charge = 0
(isoelectric form)
Net charge = –1

Figure 1.10
Ionic forms of alanine in acidic, neutral, and basic solutions.

1.9, a solution containing acetic acid (HA = $CH_3 – COOH$) and acetate ($A^- = CH_3 – COO^-$) with a pK_a of 4.8 resists a change in pH from pH 3.8 to 5.8, with maximum buffering at pH 4.8. At pH values less than the pK_a, the protonated acid form ($CH_3 – COOH$) is the predominant species in solution. At pH values greater than the pK_a, the deprotonated base form ($CH_3 – COO^-$) is the predominant species.

C. Titration of an amino acid

1. **Dissociation of the carboxyl group:** The titration curve of an amino acid can be analyzed in the same way as described for acetic acid. Consider alanine, for example, which contains an ionizable α-carboxyl and α-amino group. [Note: Its $–CH_3$ R group is nonionizable.] At a low (acidic) pH, both of these groups are protonated (shown in Figure 1.10). As the pH of the solution is raised, the – COOH group of form I can dissociate by donating a proton to the medium. The release of a proton results in the formation of the carboxylate group, $– COO^-$. This structure is shown as form II, which is the dipolar form of the molecule (see Figure 1.10). This form, also called a zwitterion, is the isoelectric form of alanine, that is, it has an overall (net) charge of zero.

2. **Application of the Henderson-Hasselbalch equation:** The dissociation constant of the carboxyl group of an amino acid is called K_1, rather than K_a, because the molecule contains a second titratable group. The Henderson-Hasselbalch equation can be used to analyze the dissociation of the carboxyl group of alanine in the same way as described for acetic acid:

$$K_1 = \frac{[H^+][II]}{[I]}$$

where I is the fully protonated form of alanine, and II is the isoelectric form of alanine (see Figure 1.10). This equation can be rearranged and converted to its logarithmic form to yield:

$$pH = pK_1 + \log \frac{[II]}{[I]}$$

3. **Dissociation of the amino group:** The second titratable group of alanine is the amino ($-NH_3^+$) group shown in Figure 1.10. This is a much weaker acid than the –COOH group and, therefore, has a much smaller dissociation constant, K_2. [Note: Its pK_a is, therefore, larger.] Release of a proton from the protonated amino group of form II results in the fully deprotonated form of alanine, form III (see Figure 1.10).

4. **pKs of alanine:** The sequential dissociation of protons from the carboxyl and amino groups of alanine is summarized in Figure 1.10. Each titratable group has a pK_a that is numerically equal to the pH at which exactly one half of the protons have been removed from that group. The pK_a for the most acidic group (–COOH) is pK_1, whereas the pK_a for the next most acidic group ($-NH_3^+$) is pK_2. [Note: The pK_a of the a-carboxyl group of amino acids is approximately 2, whereas that of the a-amino is approximately 9.]

5. **Titration curve of alanine:** By applying the Henderson-Hasselbalch equation to each dissociable acidic group, it is possible to calculate the complete titration curve of a weak acid. Figure 1.11 shows the change in pH that occurs during the addition of base to the fully protonated form of alanine (I) to produce the completely deprotonated form (III). Note the following:

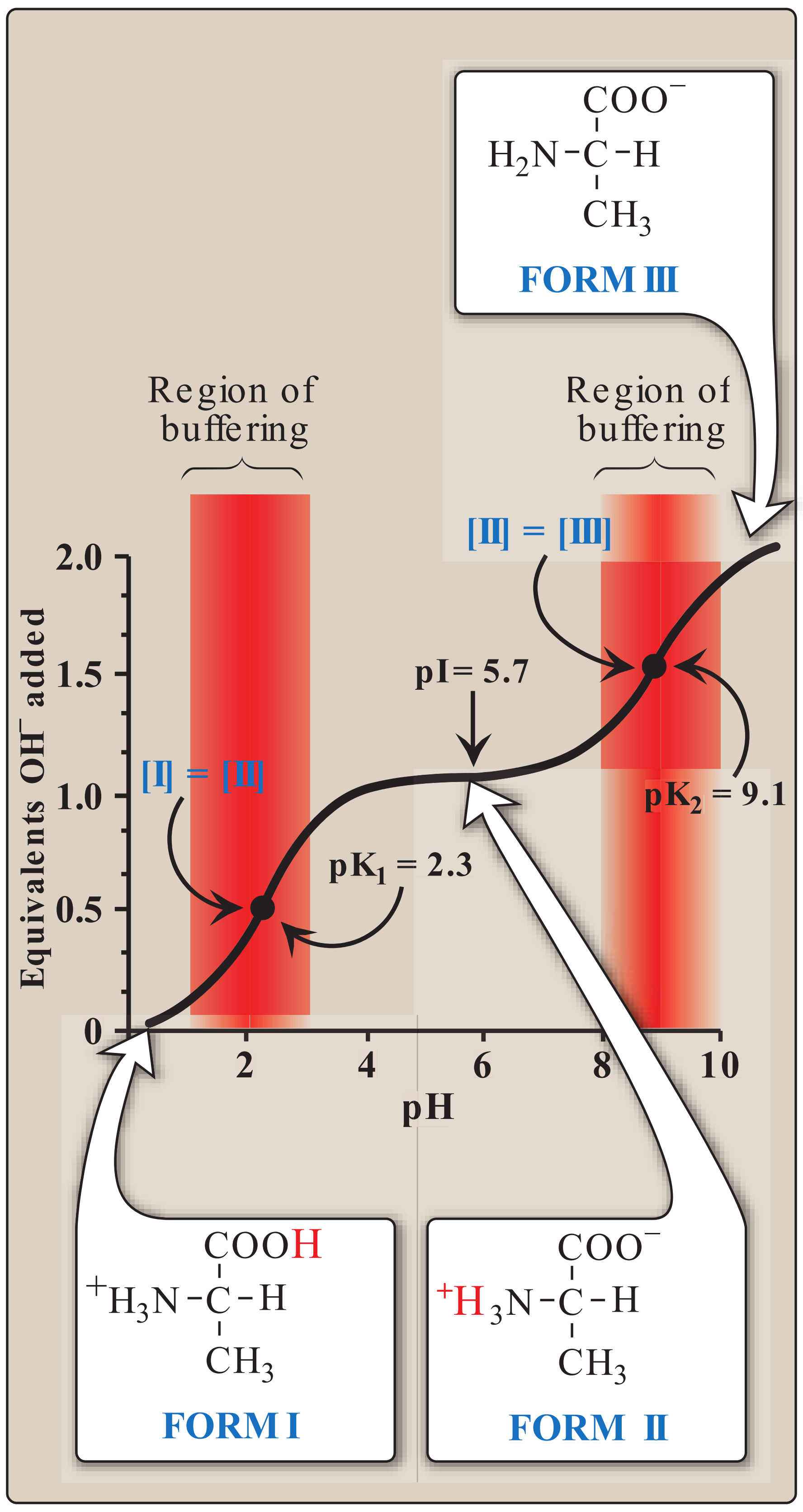


Figure 1.11
The titration curve of alanine.

   a. **Buffer pairs:** The $-COOH/-COO^-$ pair can serve as a buffer in the pH region around pK_1, and the $-NH_3^+/-NH_2$ pair can buffer in the region around pK_2.

   b. **When pH = pK:** When the pH is equal to pK_1 (2.3), equal amounts of forms I and II of alanine exist in solution. When the pH is equal to pK_2 (9.1), equal amounts of forms II and III are present in solution.

   c. **Isoelectric point:** At neutral pH, alanine exists predominantly as the dipolar form II in which the amino and carboxyl groups are ionized, but the net charge is zero. The isoelectric point (pI) is the pH at which an amino acid is electrically neutral, that is, in which the sum of the positive charges equals the sum of the negative charges. For an amino acid, such as alanine, that has only two dissociable hydrogens (one from the a-carboxyl and one from the a-amino group), the pI is the average of pK_1 and pK_2 (pI = [2.3 + 9.1]/2 = 5.7) as shown in Figure 1.11. The pI is, thus, midway between pK_1 (2.3) and pK_2 (9.1). pI corresponds to the pH at which the form II (with a net charge of zero) predominates and at which there are also equal amounts of forms I (net charge of +1) and III (net charge of –1).

> Separation of plasma proteins by charge typically is done at a pH above the pI of the major proteins. Thus, the charge on the proteins is negative. In an electric field, the proteins will move toward the positive electrode at a rate determined by their net negative charge. Variations in the mobility pattern are suggestive of certain diseases.

6. **Net charge of amino acids at neutral pH:** At physiologic pH, amino acids have a negatively charged group ($-COO^-$) and a positively charged group ($-NH_3^+$), both attached to the a-carbon. [Note: Glutamate, aspartate, histidine, arginine, and lysine have additional potentially charged groups in their side chains.] Substances such as amino acids that can act either as an acid or a base are defined as amphoteric and are referred to as ampholytes (amphoteric electrolytes).

D. Other applications of the Henderson-Hasselbalch equation

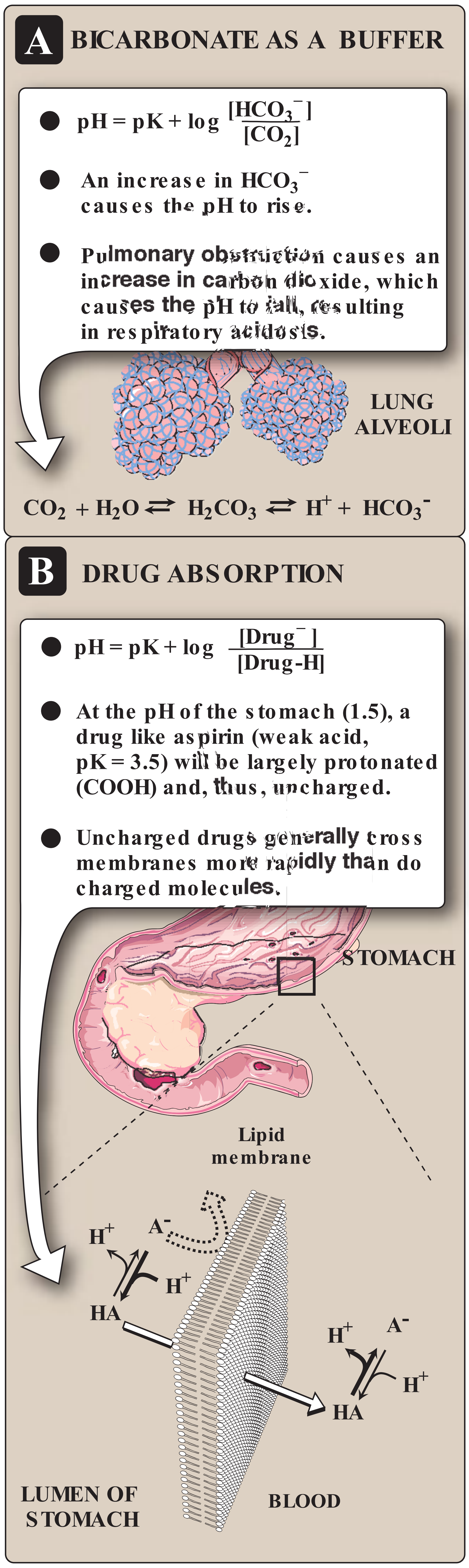
The Henderson-Hasselbalch equation can be used to calculate how the pH of a physiologic solution responds to changes in the concentration of a weak acid and/or its corresponding "salt" form. For example, in the bicarbonate buffer system, the Henderson-Hasselbalch equation predicts how shifts in the bicarbonate ion concentration, [HCO_3^-], and CO_2 influence pH (Figure 1.12A). The equation is also useful for calculating the abundance of ionic forms of acidic and basic drugs. For example, most drugs are either weak acids or weak bases (Figure 1.12B). Acidic drugs (HA) release a proton (H^+), causing a charged anion (A^-) to form.

$$HA \rightleftarrows H^+ + A^-$$

Weak bases (BH^+) can also release a H^+. However, the protonated form of basic drugs is usually charged, and the loss of a proton produces the uncharged base (B).

$$BH^+ \rightleftarrows B + H^+$$

A drug passes through membranes more readily if it is uncharged. Thus, for a weak acid, such as aspirin, the uncharged HA can permeate through membranes, but A^- cannot. For a weak base, such as morphine, the uncharged form, B, penetrates through the cell membrane, but BH^+ does not. Therefore, the effective concentration of the permeable form of each drug at its absorption site is determined by the relative concentrations of the charged (impermeant) and uncharged (permeant) forms. The ratio between the two forms is determined by the pH at the site of absorption, and by the strength of the weak acid or base, which is represented by the pK_a of the ionizable group. The Henderson-Hasselbalch equation is useful in determining how much drug is found on either side of a membrane that separates two compartments that differ in pH, for example, the stomach (pH 1.0–1.5) and blood plasma (pH 7.4).



- $pH = pK + \log \frac{[HCO_3^-]}{[CO_2]}$
- An increase in HCO_3^- causes the pH to rise.
- Pulmonary obstruction causes an increase in carbon dioxide, which causes the pH to fall, resulting in respiratory acidosis.

$$CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^-$$

- $pH = pK + \log \frac{[Drug^-]}{[Drug\text{-}H]}$
- At the pH of the stomach (1.5), a drug like aspirin (weak acid, pK = 3.5) will be largely protonated (COOH) and, thus, uncharged.
- Uncharged drugs generally cross membranes more rapidly than do charged molecules.

Figure 1.12
The Henderson-Hasselbalch equation is used to predict: A, changes in pH as the concentrations of HCO_3^- or CO_2 are altered, or B, the ionic forms of drugs.

IV. CONCEPT MAPS

Students sometimes view biochemistry as a list of facts or equations to be memorized, rather than a body of concepts to be understood. Details provided to enrich understanding of these concepts inadvertently turn into distractions. What seems to be missing is a road map—a guide that provides the student with an understanding of how various topics fit together to make sense. Therefore, a series of biochemical concept maps have been created to graphically illustrate relationships between ideas presented in a chapter and to show how the information can be grouped or organized. A concept map is, thus, a tool for visualizing the connections between concepts. Material is represented in a hierarchic fashion, with the most inclusive, most general concepts at the top of the map and the more specific, less general concepts arranged beneath. The concept maps ideally function as templates or guides for organizing information, so the student can readily find the best ways to integrate new information into knowledge they already possess.

A. How is a concept map constructed?

1. **Concept boxes and links:** Educators define concepts as "perceived regularities in events or objects." In the biochemical maps, concepts include abstractions (for example, free energy), processes (for example, oxidative phosphorylation), and compounds (for example, glucose 6-phosphate). These broadly defined concepts are prioritized with the central idea positioned at the top of the page. The concepts that follow from this central idea are then drawn in boxes (Figure 1.13A). The size of the type indicates the relative importance of each idea. Lines are drawn between concept boxes to show which are related. The label on the line defines the relationship between two concepts, so that it reads as a valid statement, that is, the connection creates meaning. The lines with arrowheads indicate in which direction the connection should be read (Figure 1.14).

2. **Cross-links:** Unlike linear flow charts or outlines, concept maps may contain cross-links that allow the reader to visualize complex relationships between ideas represented in different parts of the map (Figure 1.13B), or between the map and other chapters in this book (Figure 1.13C). Cross-links can, thus, identify concepts that are central to more than one topic in biochemistry, empowering students to be effective in clinical situations and on the United States Medical Licensure Examination (USMLE) or other examinations that require integration of material. Students learn to visually perceive nonlinear relationships between facts, in contrast to cross-referencing within linear text.

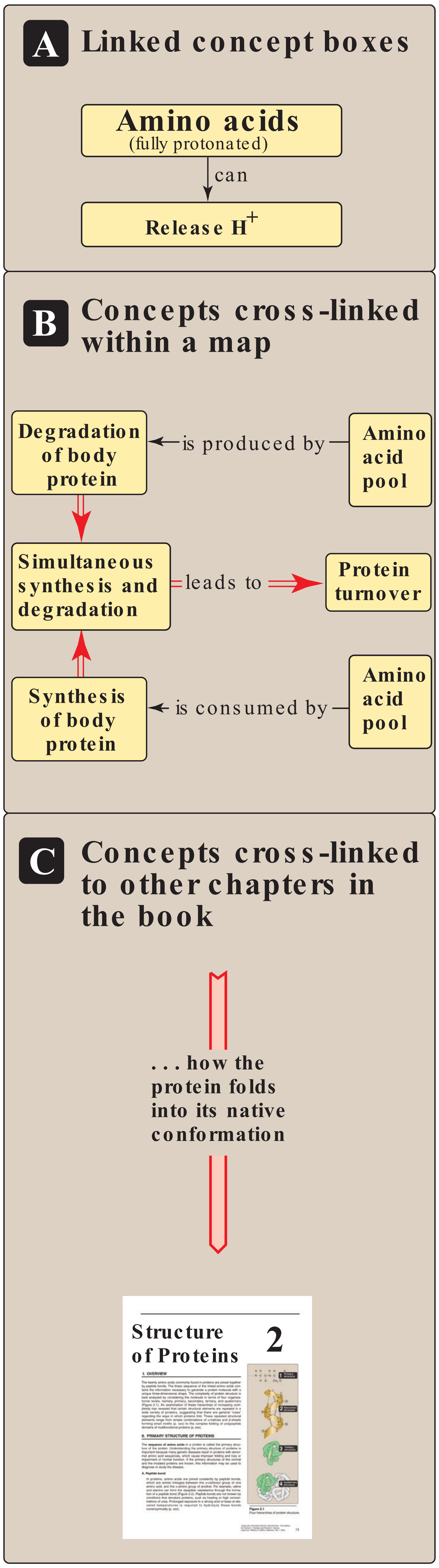

A
Linked concept boxes
Amino acids
(fully protonated)
can
Release H+
B
Concepts cross-linked within a map
Degradation of body protein
is produced by
Amino acid pool
Simultaneous synthesis and degradation
leads to
Protein turnover
Synthesis of body protein
is consumed by
Amino acid pool
C
Concepts cross-linked to other chapters in the book
. . . how the protein folds into its native conformation
Structure of Proteins
2

Figure 1.13
Symbols used in concept maps.

V. CHAPTER SUMMARY

Each amino acid has an **α-carboxyl group** and a primary **α-amino group** (except for proline, which has a **secondary amino group**). At physiologic pH, the α-carboxyl group is dissociated, forming the negatively charged carboxylate ion ($-COO^-$), and the α-amino group is protonated ($-NH_3^+$). Each amino acid also contains one of 20 distinctive

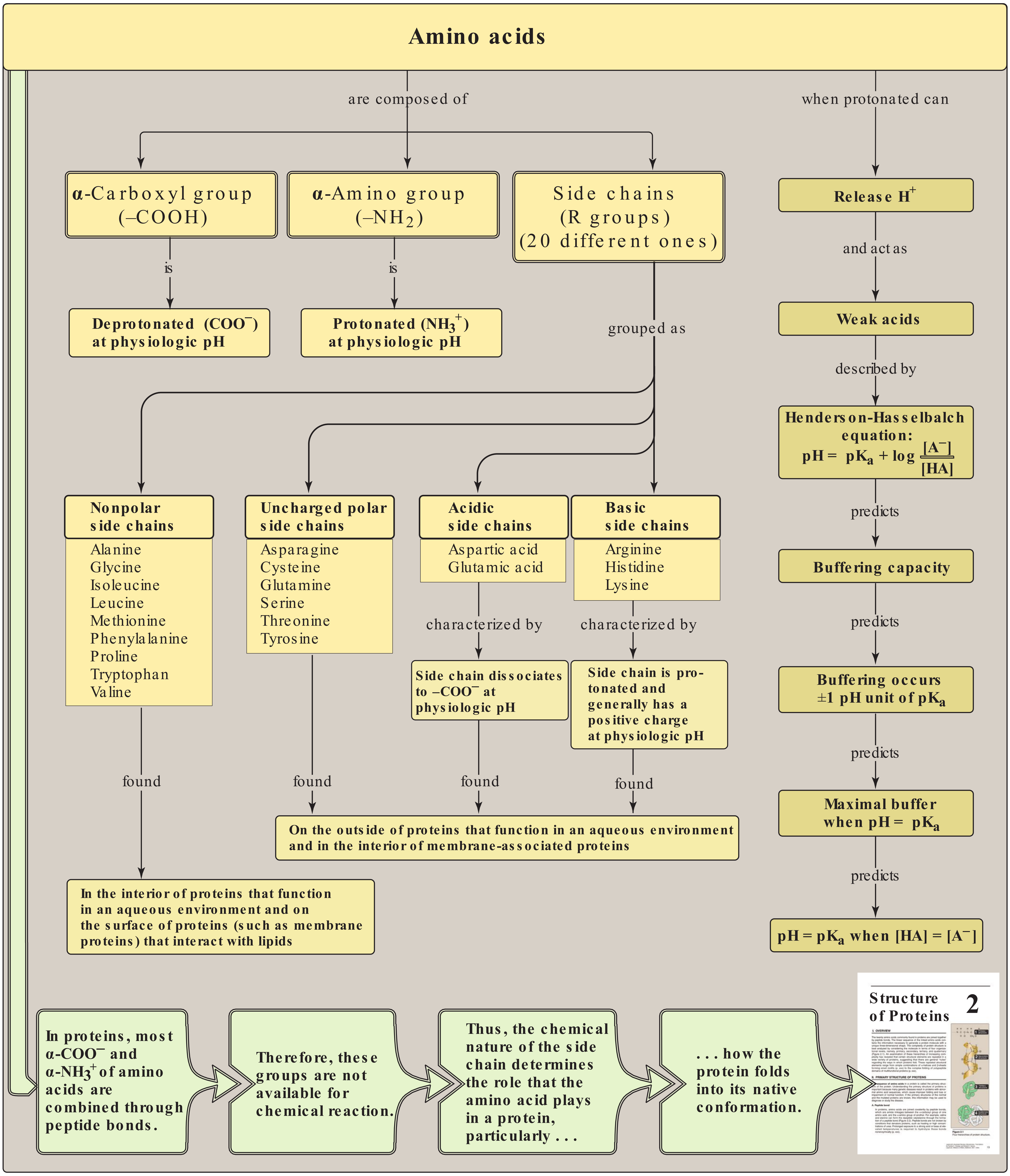

Amino acids
are composed of
when protonated can
α-Carboxyl group (–COOH)
α-Amino group (–NH_2)
Side chains (R groups) (20 different ones)
is
is
Deprotonated (COO^-) at physiologic pH
Protonated (NH_3^+) at physiologic pH
grouped as
Release H^+
and act as
Weak acids
described by
Henderson-Hasselbalch equation: $pH = pK_a + \log \frac{[A^-]}{[HA]}$
predicts
Buffering capacity
predicts
Buffering occurs ±1 pH unit of pK_a
predicts
Maximal buffer when $pH = pK_a$
predicts
$pH = pK_a$ when $[HA] = [A^-]$
Nonpolar side chains
Alanine
Glycine
Isoleucine
Leucine
Methionine
Phenylalanine
Proline
Tryptophan
Valine
Uncharged polar side chains
Asparagine
Cysteine
Glutamine
Serine
Threonine
Tyrosine
Acidic side chains
Aspartic acid
Glutamic acid
Basic side chains
Arginine
Histidine
Lysine
characterized by
characterized by
Side chain dissociates to $-COO^-$ at physiologic pH
Side chain is protonated and generally has a positive charge at physiologic pH
found
found
found
found
On the outside of proteins that function in an aqueous environment and in the interior of membrane-associated proteins
In the interior of proteins that function in an aqueous environment and on the surface of proteins (such as membrane proteins) that interact with lipids
In proteins, most α-COO^- and α-NH_3^+ of amino acids are combined through peptide bonds.
Therefore, these groups are not available for chemical reaction.
Thus, the chemical nature of the side chain determines the role that the amino acid plays in a protein, particularly . . .
. . . how the protein folds into its native conformation.
Structure of Proteins
2

Figure 1.14
Key concept map for amino acids.

side chains attached to the a-carbon atom. The chemical nature of this R group determines the function of an amino acid in a protein and provides the basis for classification of the amino acids as **nonpolar**, **uncharged polar**, **acidic** (**polar negative**), or **basic** (**polar positive**). All free amino acids, plus charged amino acids in peptide chains, can serve as **buffers**. The quantitative relationship between the pH of a solution and the concentration of a weak acid (HA) and its conjugate base (A^-) is described by the **Henderson-Hasselbalch equation**. Buffering occurs within ±1 pH unit of the pK_a and is maximal when $pH = pK_a$, at which $[A^-] = [HA]$. The a-carbon of each amino acid (except glycine) is attached to four different chemical groups and is, therefore, a **chiral**, or **optically active** carbon atom. The L-form of amino acids is found in proteins synthesized by the human body.

Structure of Proteins

2

I. OVERVIEW

The 20 amino acids commonly found in proteins are joined together by peptide bonds. The linear sequence of the linked amino acids contains the information necessary to generate a protein molecule with a unique three-dimensional shape. The complexity of protein structure is best analyzed by considering the molecule in terms of four organizational levels: primary, secondary, tertiary, and quaternary (Figure 2.1). An examination of these hierarchies of increasing complexity has revealed that certain structural elements are repeated in a wide variety of proteins, suggesting that there are general "rules" regarding the ways in which proteins achieve their native, functional form. These repeated structural elements range from simple combinations of α-helices and β-sheets forming small motifs, to the complex folding of polypeptide domains of multifunctional proteins (see p. 19).

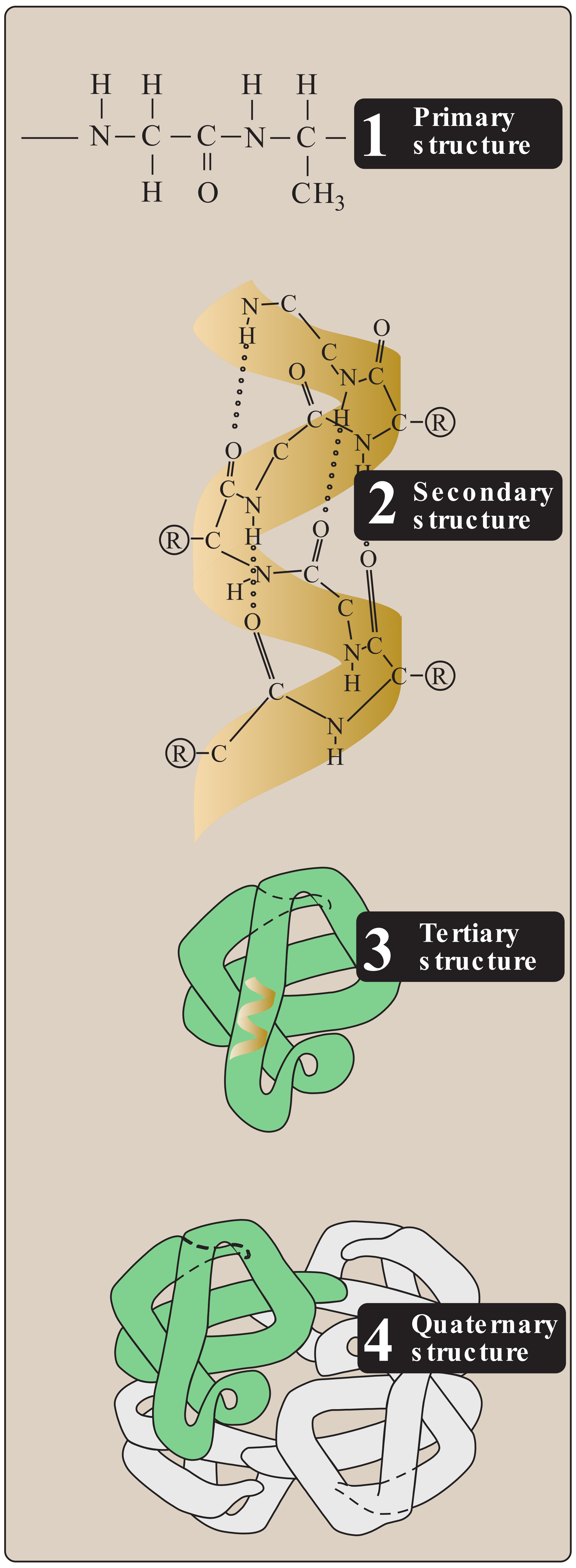


Figure 2.1
Four hierarchies of protein structure.

II. PRIMARY STRUCTURE OF PROTEINS

The sequence of amino acids in a protein is called the primary structure of the protein. Understanding the primary structure of proteins is important because many genetic diseases result in proteins with abnormal amino acid sequences, which cause improper folding and loss or impairment of normal function. If the primary structures of the normal and the mutated proteins are known, this information may be used to diagnose or study the disease.

A. Peptide bond

In proteins, amino acids are joined covalently by peptide bonds, which are amide linkages between the α-carboxyl group of one amino acid and the α-amino group of another. For example, valine and alanine can form the dipeptide valylalanine through the formation of a peptide bond (Figure 2.2). Peptide bonds are resistant to conditions that denature proteins, such as heating and high concentrations of urea (see p. 20). Prolonged exposure to a strong acid or base at elevated temperatures is required to break these bonds nonenzymically.

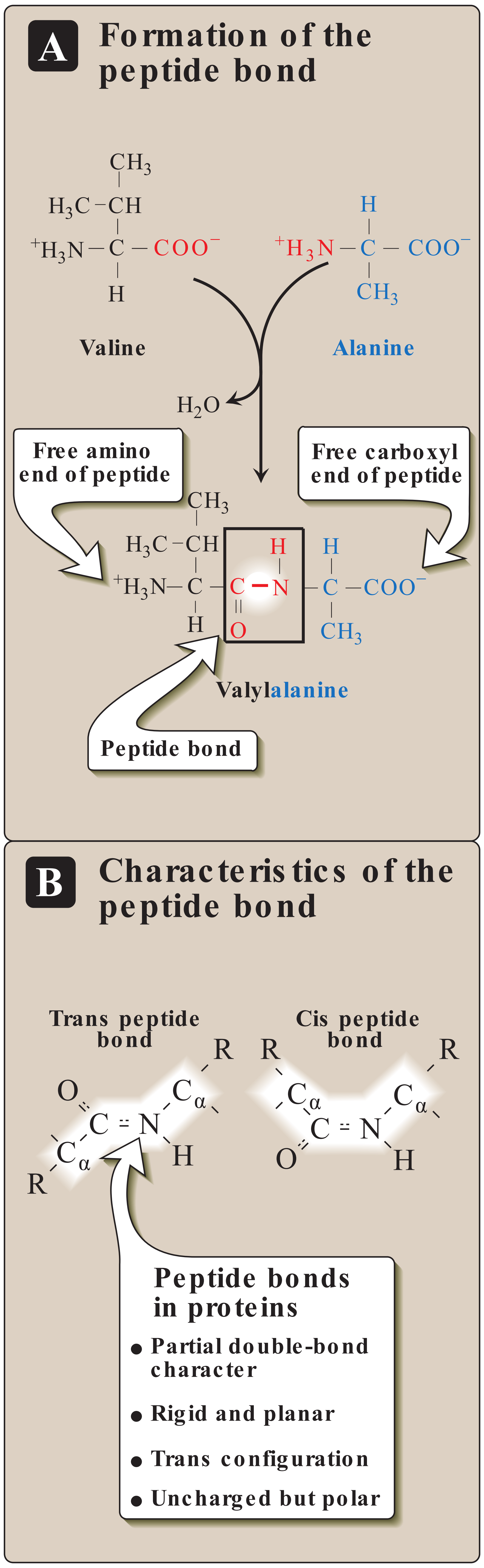

A
Formation of the peptide bond
Valine
Alanine
H2O
Free amino end of peptide
Free carboxyl end of peptide
Valylalanine
Peptide bond
B
Characteristics of the peptide bond
Trans peptide bond
Cis peptide bond
Peptide bonds in proteins
Partial double-bond character
Rigid and planar
Trans configuration
Uncharged but polar

Figure 2.2
A. Formation of a peptide bond, showing the structure of the dipeptide valylalanine.
B. Characteristics of the peptide bond.

1. **Naming the peptide:** By convention, the free amino end (N-terminal) of the peptide chain is written to the left and the free carboxyl end (C-terminal) to the right. Therefore, all amino acid sequences are read from the N- to the C-terminal end of the peptide. For example, in Figure 2.2A, the order of the amino acids is "valine, alanine." Linkage of many amino acids through peptide bonds results in an unbranched chain called a polypeptide. Each component amino acid in a polypeptide is called a "residue" because it is the portion of the amino acid remaining after the atoms of water are lost in the formation of the peptide bond. When a polypeptide is named, all amino acid residues have their suffixes (-ine, -an, -ic, or -ate) changed to -yl, with the exception of the C-terminal amino acid. For example, a tripeptide composed of an N-terminal valine, a glycine, and a C-terminal leucine is called valylglycylleucine.

2. **Characteristics of the peptide bond:** The peptide bond has a partial double-bond character, that is, it is shorter than a single bond and is rigid and planar (Figure 2.2B). This prevents free rotation around the bond between the carbonyl carbon and the nitrogen of the peptide bond. However, the bonds between the α-carbons and the α-amino or α-carboxyl groups can be freely rotated (although they are limited by the size and character of the R groups). This allows the polypeptide chain to assume a variety of possible configurations. The peptide bond is almost always a trans bond (instead of cis, see Figure 2.2B), in large part because of steric interference of the R groups when in the cis position.

3. **Polarity of the peptide bond:** Like all amide linkages, the $-C{=}O$ and $-NH$ groups of the peptide bond are uncharged and neither accept nor release protons over the pH range of 2–12. Thus, the charged groups present in polypeptides consist solely of the N-terminal (α-amino) group, the C-terminal (α-carboxyl) group, and any ionized groups present in the side chains of the constituent amino acids. The $-C{=}O$ and $-NH$ groups of the peptide bond are polar, however, and are involved in hydrogen bonds (for example, in α-helices and β-sheets), as described on pp. 16–17.

B. Determination of the amino acid composition of a polypeptide

The first step in determining the primary structure of a polypeptide is to identify and quantitate its constituent amino acids. A purified sample of the polypeptide to be analyzed is first hydrolyzed by strong acid at 110°C for 24 hours. This treatment cleaves the peptide bonds and releases the individual amino acids, which can be separated by cation-exchange chromatography. In this technique, a mixture of amino acids is applied to a column that contains a resin to which a negatively charged group is tightly attached. [Note: If the attached group is positively charged, the column becomes an anion-exchange column.] The amino acids bind to the column with different affinities, depending on their charges, hydrophobicity, and other characteristics. Each amino acid is sequentially released from the chromatography column by eluting with solutions of increasing ionic strength and pH (Figure 2.3). The separated amino acids contained in the eluate from the column are quantitated by heating them with ninhydrin (a reagent that forms a purple compound with most amino acids, ammonia, and amines). The amount of each amino acid is determined spectrophotometrically by measuring the amount of light absorbed by the ninhydrin derivative. The analysis described above is performed using an amino acid analyzer, an automated machine whose components are depicted in Figure 2.3.

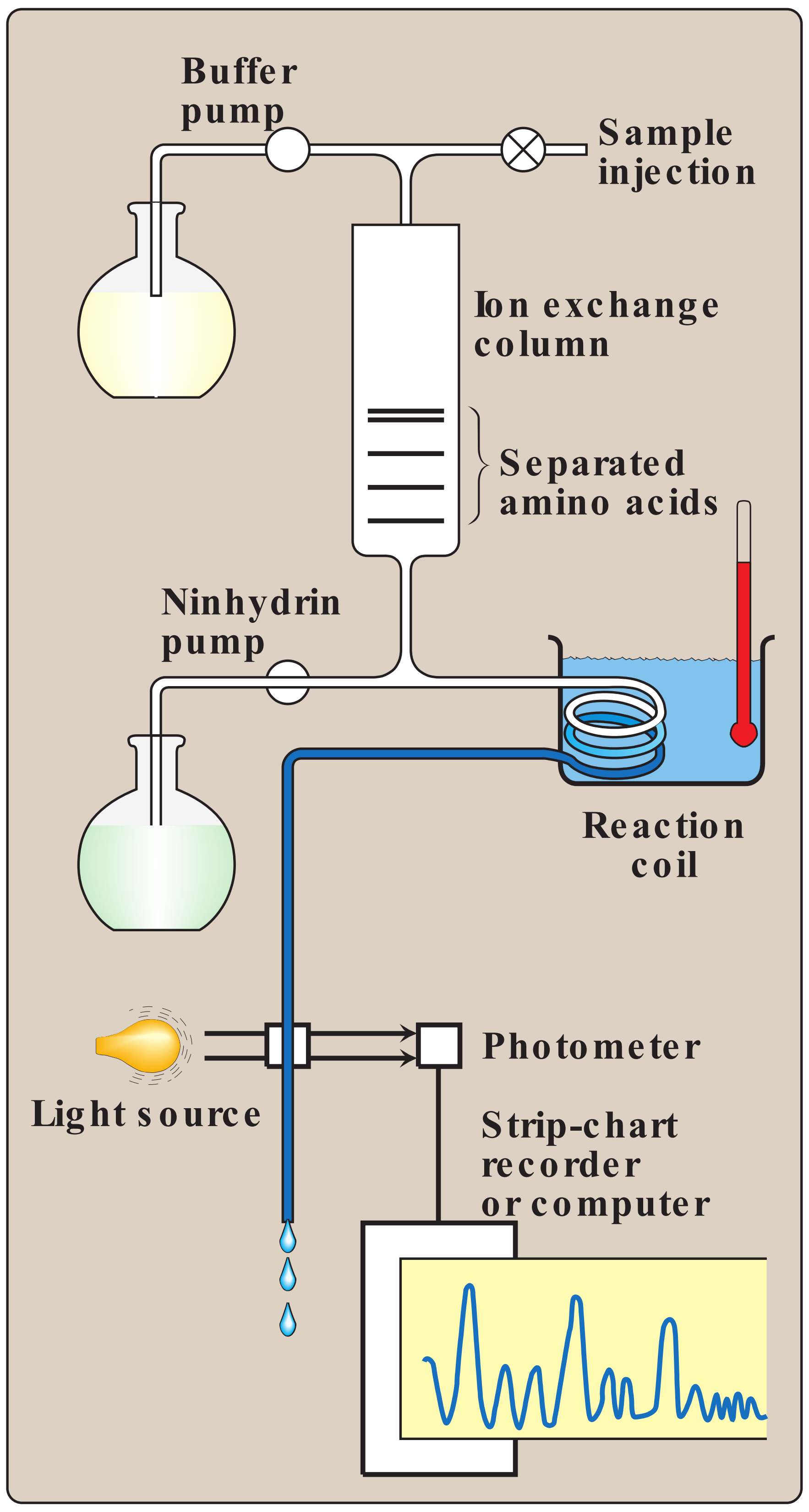

Buffer pump
Sample injection
Ion exchange column
Separated amino acids
Ninhydrin pump
Reaction coil
Light source
Photometer
Strip-chart recorder or computer

Figure 2.3
Determination of the amino acid composition of a polypeptide using an amino acid analyzer.

C. Sequencing of the peptide from its N-terminal end

Sequencing is a stepwise process of identifying the specific amino acid at each position in the peptide chain, beginning at the N-terminal end. Phenylisothiocyanate, known as Edman reagent, is used to label the amino-terminal residue under mildly alkaline conditions (Figure 2.4). The resulting phenylthiohydantoin (PTH) derivative introduces an instability in the N-terminal peptide bond such that it can be hydrolyzed without cleaving the other peptide bonds. The identity of the amino acid derivative can then be determined. Edman reagent can be applied repeatedly to the shortened peptide obtained in each previous cycle. The process is now automated.

D. Cleavage of the polypeptide into smaller fragments

Many polypeptides have a primary structure composed of more than 100 amino acids. Such molecules cannot be sequenced directly from end to end. However, these large molecules can be cleaved at specific sites and the resulting fragments sequenced. By using more than one cleaving agent (enzymes and/or chemicals) on separate samples of the purified polypeptide, overlapping fragments can be generated that permit the proper ordering of the sequenced fragments, thereby providing a complete amino acid sequence of the large polypeptide (Figure 2.5). Enzymes that hydrolyze peptide bonds are termed peptidases (proteases). [Note: Exopeptidases cut at the ends of proteins and are divided into aminopeptidases and carboxypeptidases. Carboxypeptidases are used in determining the C-terminal amino acid. Endopeptidases cleave within a protein.]

E. Determination of a protein's primary structure by DNA sequencing

The sequence of nucleotides in a protein-coding region of the DNA specifies the amino acid sequence of a polypeptide. Therefore, if the nucleotide sequence can be determined, it is possible, from knowledge of the genetic code (see p. 432), to translate the sequence of nucleotides into the corresponding amino acid sequence of that polypeptide. This indirect process, although routinely used to

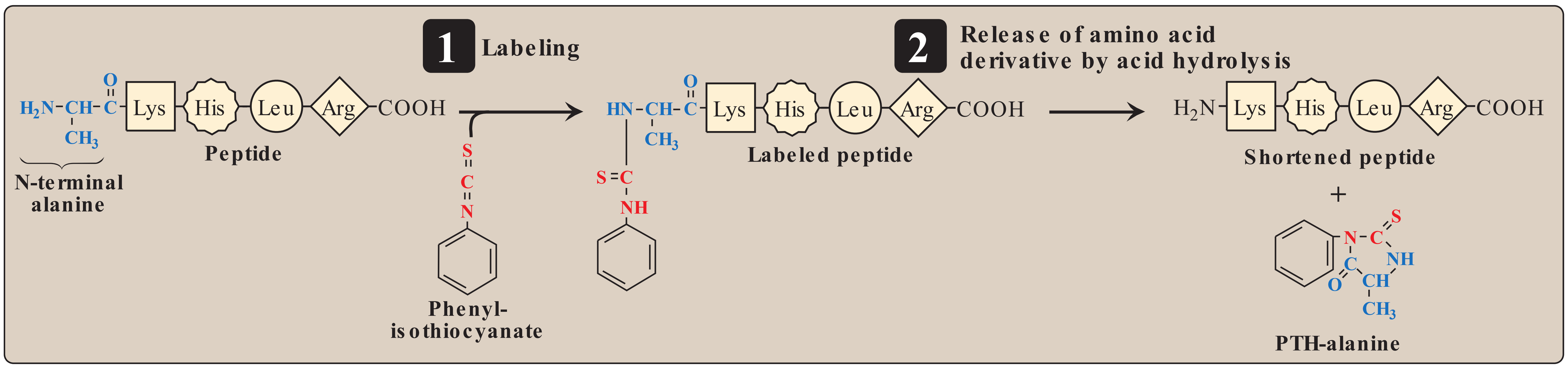

1 Labeling
2 Release of amino acid derivative by acid hydrolysis
H2N-CH-C
O
CH3
Lys
His
Leu
Arg
COOH
N-terminal alanine
Peptide
S
C
N
Phenyl-isothiocyanate
HN-CH-C
S=C
NH
Labeled peptide
H2N
Shortened peptide
+
N-C
S
NH
C
O
CH
CH3
PTH-alanine

Figure 2.4
Determination of the amino (N)-terminal residue of a polypeptide by Edman degradation. PTH = phenylthiohydantoin.

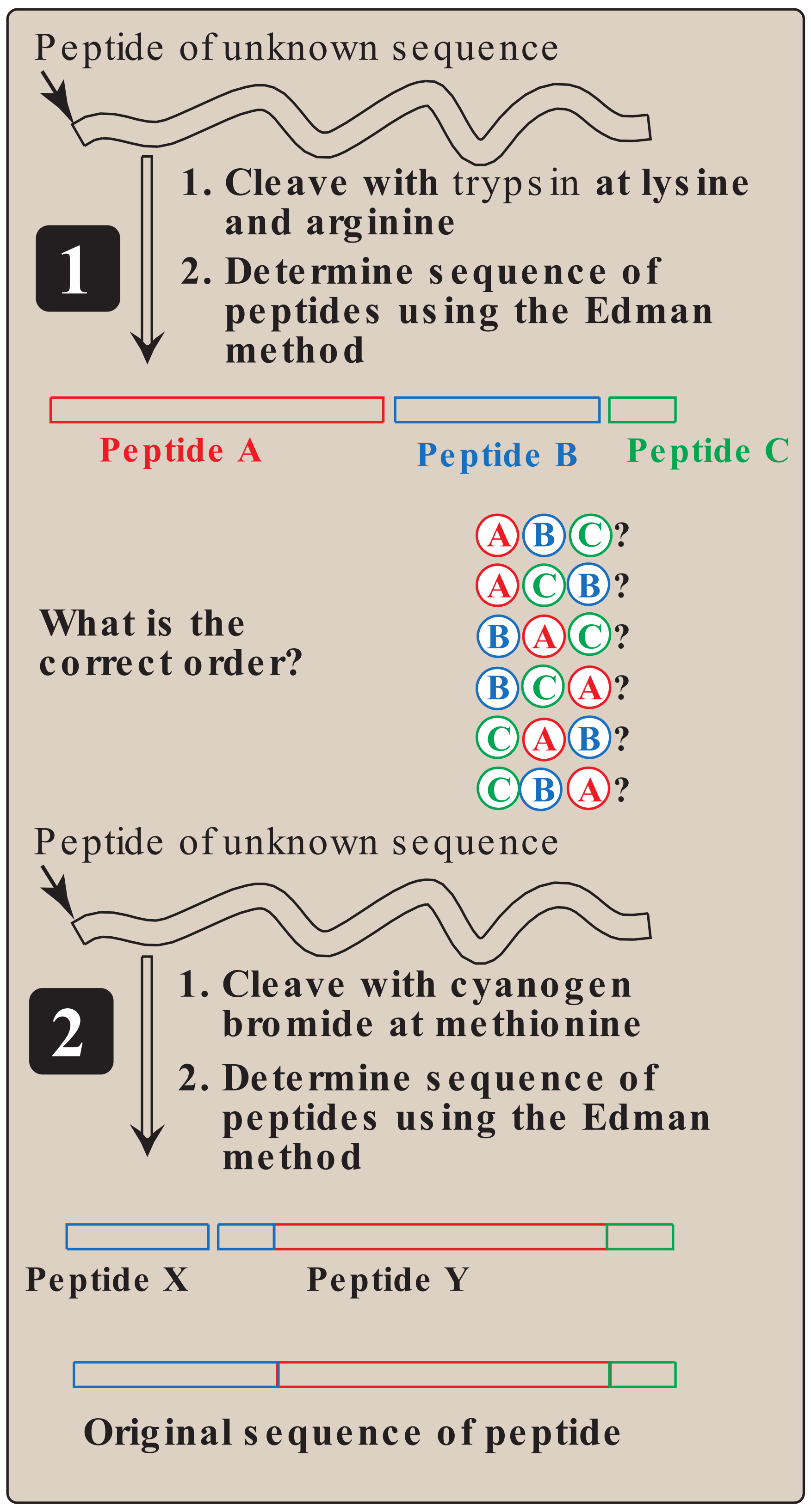

Peptide of unknown sequence
1
1. Cleave with trypsin at lysine and arginine
2. Determine sequence of peptides using the Edman method
Peptide A
Peptide B
Peptide C
What is the correct order?
ABC?
ACB?
BAC?
BCA?
CAB?
CBA?
Peptide of unknown sequence
2
1. Cleave with cyanogen bromide at methionine
2. Determine sequence of peptides using the Edman method
Peptide X
Peptide Y
Original sequence of peptide

Figure 2.5
Overlapping of peptides produced by the action of trypsin and cyanogen bromide.

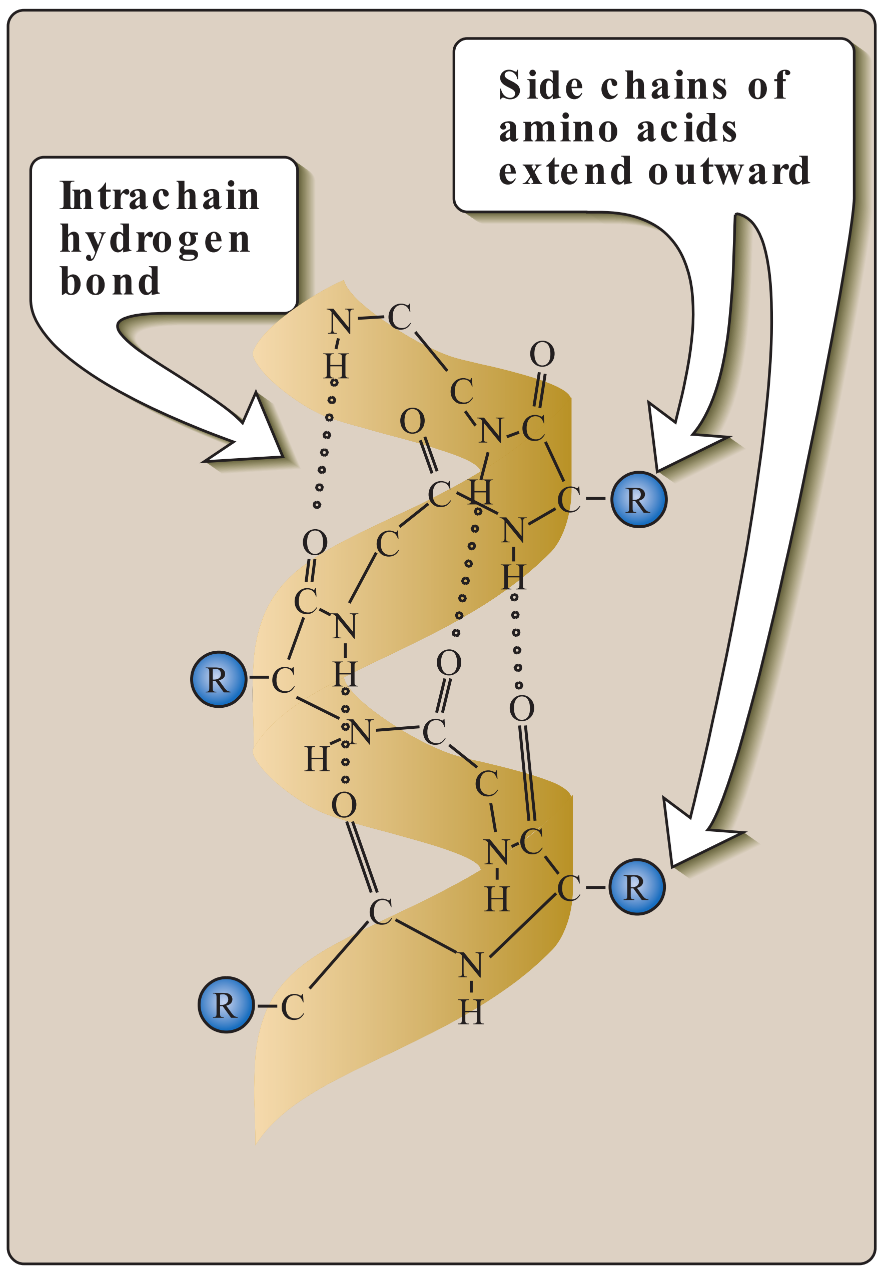

Intrachain hydrogen bond
Side chains of amino acids extend outward
R
R
R
R

Figure 2.6
α-Helix showing peptide backbone.

obtain the amino acid sequences of proteins, has the limitations of not being able to predict the positions of disulfide bonds in the folded chain and of not identifying any amino acids that are modified after their incorporation into the polypeptide (posttranslational modification, see p. 443). Therefore, direct protein sequencing is an extremely important tool for determining the true character of the primary sequence of many polypeptides.

III. SECONDARY STRUCTURE OF PROTEINS

The polypeptide backbone does not assume a random three-dimensional structure but, instead, generally forms regular arrangements of amino acids that are located near each other in the linear sequence. These arrangements are termed the secondary structure of the polypeptide. The α-helix, β-sheet, and β-bend (β-turn) are examples of secondary structures commonly encountered in proteins. [Note: The collagen α-chain helix, another example of secondary structure, is discussed on p. 45.]

A. α-Helix

Several different polypeptide helices are found in nature, but the α-helix is the most common. It is a spiral structure, consisting of a tightly packed, coiled polypeptide backbone core, with the side chains of the component amino acids extending outward from the central axis to avoid interfering sterically with each other (Figure 2.6). A very diverse group of proteins contains α-helices. For example, the keratins are a family of closely related, fibrous proteins whose structure is nearly entirely α-helical. They are a major component of tissues such as hair and skin, and their rigidity is determined by the number of disulfide bonds between the constituent polypeptide chains. In contrast to keratin, myoglobin, whose structure is also highly α-helical, is a globular, flexible molecule (see p. 26).

1. **Hydrogen bonds:** An α-helix is stabilized by extensive hydrogen bonding between the peptide-bond carbonyl oxygens and amide hydrogens that are part of the polypeptide backbone (see Figure 2.6). The hydrogen bonds extend up and are parallel to the spiral from the carbonyl oxygen of one peptide bond to the –NH– group of a peptide linkage four residues ahead in the polypeptide. This insures that all but the first and last peptide bond components are linked to each other through intrachain hydrogen bonds. Hydrogen bonds are individually weak, but they collectively serve to stabilize the helix.

2. **Amino acids per turn:** Each turn of an α-helix contains 3.6 amino acids. Thus, amino acid residues spaced three or four residues apart in the primary sequence are spatially close together when folded in the α-helix.

3. **Amino acids that disrupt an α-helix:** Proline disrupts an α-helix because its secondary amino group is not geometrically compatible with the right-handed spiral of the α-helix. Instead, it inserts a kink in the chain, which interferes with the smooth, helical structure. Large numbers of charged amino acids (for example,

glutamate, aspartate, histidine, lysine, and arginine) also disrupt the helix by forming ionic bonds or by electrostatically repelling each other. Finally, amino acids with bulky side chains, such as tryptophan, or amino acids, such as valine or isoleucine, that branch at the β-carbon (the first carbon in the R group, next to the α-carbon) can interfere with formation of the α-helix if they are present in large numbers.

B. β-Sheet

The β-sheet is another form of secondary structure in which all of the peptide bond components are involved in hydrogen bonding (Figure 2.7A). The surfaces of β-sheets appear "pleated," and these structures are, therefore, often called β-pleated sheets. When illustrations are made of protein structure, β-strands are often visualized as broad arrows (Figure 2.7B).

1. **Comparison of a β-sheet and an α-helix:** Unlike the α-helix, β-sheets are composed of two or more peptide chains (β-strands), or segments of polypeptide chains, which are almost fully extended. Note also that the hydrogen bonds are perpendicular to the polypeptide backbone in β-sheets (see Figure 2.7A).

2. **Parallel and antiparallel sheets:** A β-sheet can be formed from two or more separate polypeptide chains or segments of polypeptide chains that are arranged either antiparallel to each other (with the N-terminal and C-terminal ends of the β-strands alternating as shown in Figure 2.7B) or parallel to each other (with all the N-termini of the β-strands together as shown in Figure 2.7C). When the hydrogen bonds are formed between the polypeptide backbones of separate polypeptide chains, they are termed interchain bonds. A β-sheet can also be formed by a single polypeptide chain folding back on itself (see Figure 2.7C). In this case, the hydrogen bonds are intrachain bonds. In globular proteins, β-sheets always have a right-handed curl, or twist, when viewed along the polypeptide backbone. [Note: Twisted β-sheets often form the core of globular proteins.]

> The α-helix and β-sheet structures provide maximal hydrogen bonding for peptide bond components within the interior of polypeptides.

C. β-Bends (reverse turns, β-turns)

β-Bends reverse the direction of a polypeptide chain, helping it form a compact, globular shape. They are usually found on the surface of protein molecules and often include charged residues. [Note: β-Bends were given this name because they often connect successive strands of antiparallel β-sheets.] β-Bends are generally composed of four amino acids, one of which may be proline, the amino acid that causes a kink in the polypeptide chain. Glycine, the amino acid with the smallest R group, is also frequently found in β-bends. β-Bends are stabilized by the formation of hydrogen and ionic bonds.

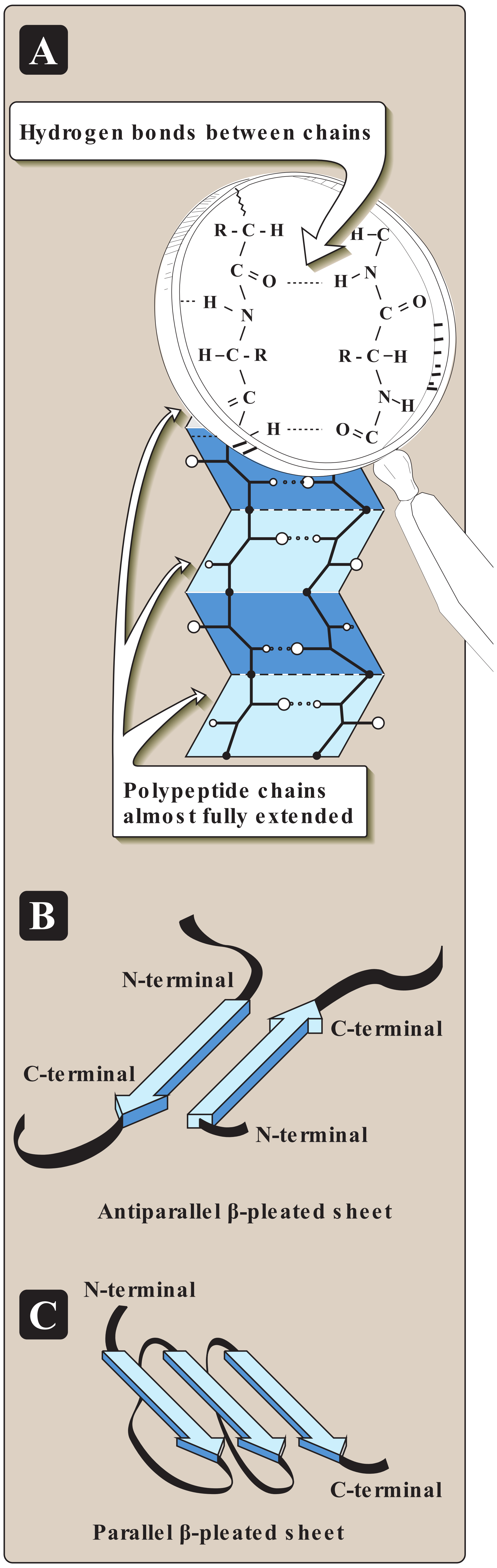


Figure 2.7
A. Structure of a β-sheet. B. An antiparallel β-sheet with the β-strands represented as broad arrows. C. A parallel β-sheet formed from a single polypeptide chain folding back on itself.

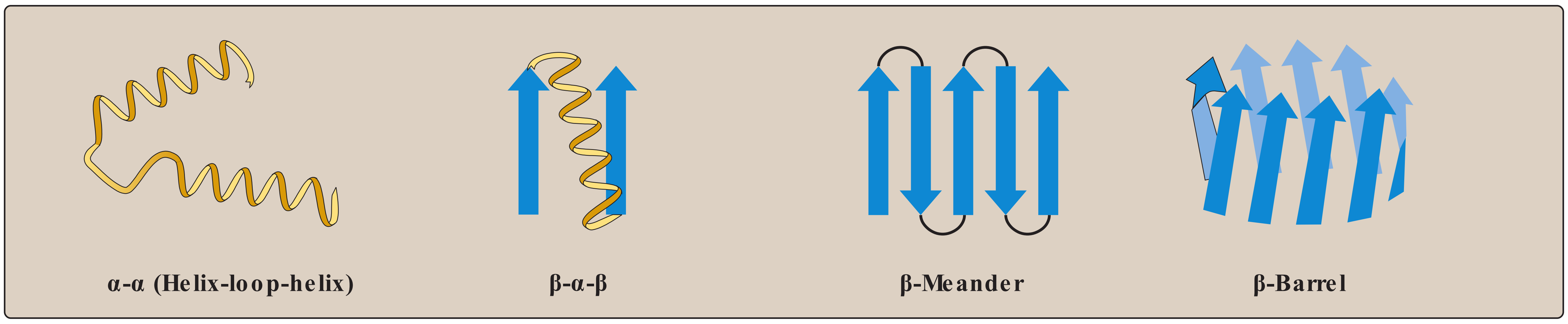


Figure 2.8
Some common structural motifs involving α-helices and β-sheets. The names describe their schematic appearance.

D. Nonrepetitive secondary structure

Approximately one half of an average globular protein is organized into repetitive structures, such as the α-helix and β-sheet. The remainder of the polypeptide chain is described as having a loop or coil conformation. These nonrepetitive secondary structures are not random, but rather simply have a less regular structure than those described above. [Note: The term "random coil" refers to the disordered structure obtained when proteins are denatured (see p. 20).]

E. Supersecondary structures (motifs)

Globular proteins are constructed by combining secondary structural elements (that is, α-helices, β-sheets, and coils), producing specific geometric patterns or motifs. These form primarily the core (interior) region of the molecule. They are connected by loop regions (for example, β-bends) at the surface of the protein. Supersecondary structures are usually produced by the close packing of side chains from adjacent secondary structural elements. Thus, for example, α-helices and β-sheets that are adjacent in the amino acid sequence are also usually (but not always) adjacent in the final, folded protein. Some of the more common motifs are illustrated in Figure 2.8.

> Motifs may be associated with particular functions. Proteins that bind to DNA contain a limited number of motifs. The helix-loop-helix motif is an example found in a number of proteins that function as transcription factors (see p. 450).

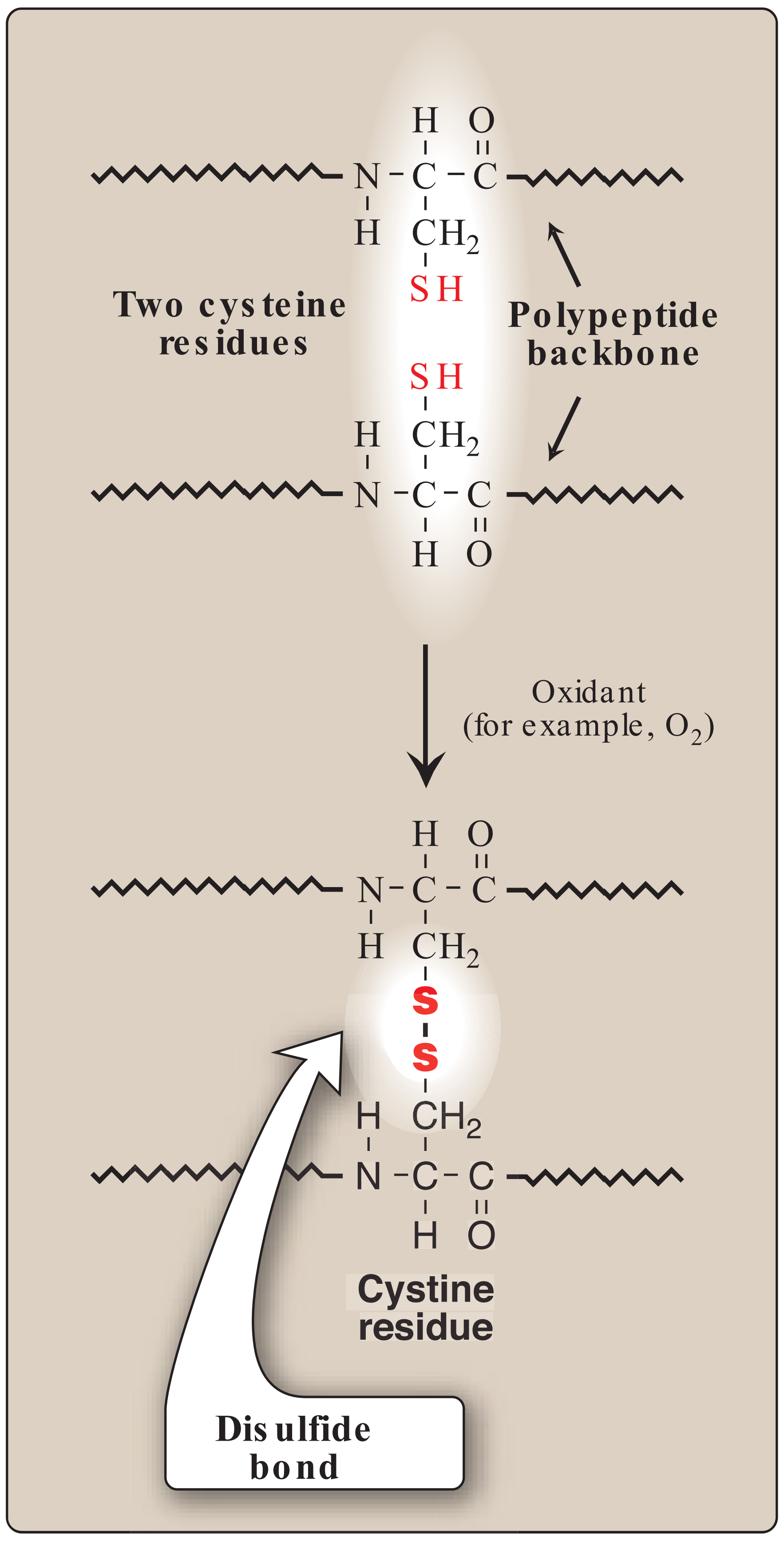


Figure 2.9
Formation of a disulfide bond by the oxidation of two cysteine residues, producing one cystine residue.

IV. TERTIARY STRUCTURE OF GLOBULAR PROTEINS

The primary structure of a polypeptide chain determines its tertiary structure. "Tertiary" refers both to the folding of domains (the basic units of structure and function, see discussion below), and to the final arrangement of domains in the polypeptide. The structure of globular proteins in aqueous solution is compact, with a high density (close packing) of the atoms in the core of the molecule. Hydrophobic side chains are buried in the interior, whereas hydrophilic groups are generally found on the surface of the molecule.

A. Domains

Domains are the fundamental functional and three-dimensional structural units of polypeptides. Polypeptide chains that are greater than 200 amino acids in length generally consist of two or more domains. The core of a domain is built from combinations of supersecondary structural elements (motifs). Folding of the peptide chain within a domain usually occurs independently of folding in other domains. Therefore, each domain has the characteristics of a small, compact globular protein that is structurally independent of the other domains in the polypeptide chain.

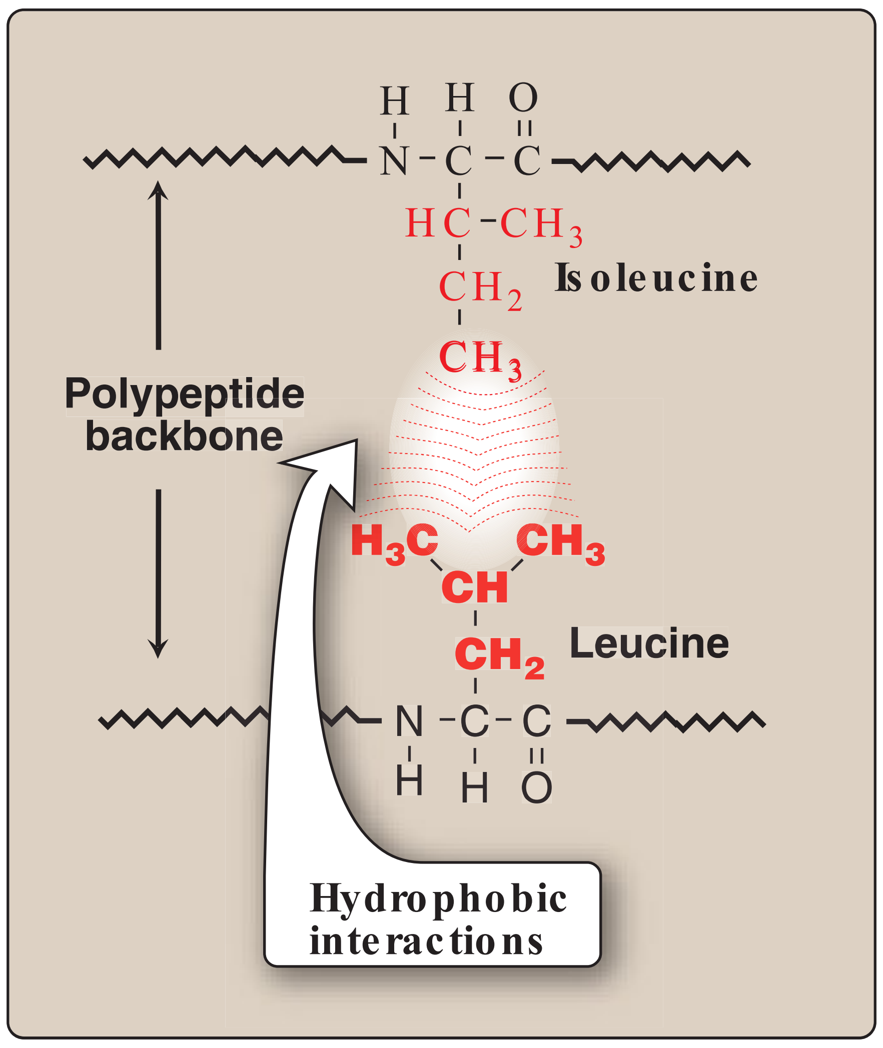


Figure 2.10
Hydrophobic interactions between amino acids with nonpolar side chains.

B. Interactions stabilizing tertiary structure

The unique three-dimensional structure of each polypeptide is determined by its amino acid sequence. Interactions between the amino acid side chains guide the folding of the polypeptide to form a compact structure. The following four types of interactions cooperate in stabilizing the tertiary structures of globular proteins.

1. **Disulfide bonds:** A disulfide bond is a covalent linkage formed from the sulfhydryl group (–SH) of each of two cysteine residues to produce a cystine residue (Figure 2.9). The two cysteines may be separated from each other by many amino acids in the primary sequence of a polypeptide or may even be located on two different polypeptide chains. The folding of the polypeptide chain(s) brings the cysteine residues into proximity and permits covalent bonding of their side chains. A disulfide bond contributes to the stability of the three-dimensional shape of the protein molecule and prevents it from becoming denatured in the extracellular environment. For example, many disulfide bonds are found in proteins such as immunoglobulins that are secreted by cells.

2. **Hydrophobic interactions:** Amino acids with nonpolar side chains tend to be located in the interior of the polypeptide molecule, where they associate with other hydrophobic amino acids (Figure 2.10). In contrast, amino acids with polar or charged side chains tend to be located on the surface of the molecule in contact with the polar solvent. [Note: Recall that proteins located in nonpolar (lipid) environments, such as a membrane, exhibit the reverse arrangement (see Figure 1.4, p. 4).] In each case, a segregation of R groups occurs that is energetically most favorable.

3. **Hydrogen bonds:** Amino acid side chains containing oxygen- or nitrogen-bound hydrogen, such as in the alcohol groups of serine and threonine, can form hydrogen bonds with electron-rich atoms, such as the oxygen of a carboxyl group or carbonyl group of a peptide bond (Figure 2.11; see also Figure 1.6, p. 4). Formation of hydrogen bonds between polar groups on the surface of proteins and the aqueous solvent enhances the solubility of the protein.

4. **Ionic interactions:** Negatively charged groups, such as the carboxylate group (– COO$^-$) in the side chain of aspartate or glutamate, can interact with positively charged groups such as the amino group (– NH$_3^+$) in the side chain of lysine (see Figure 2.11).

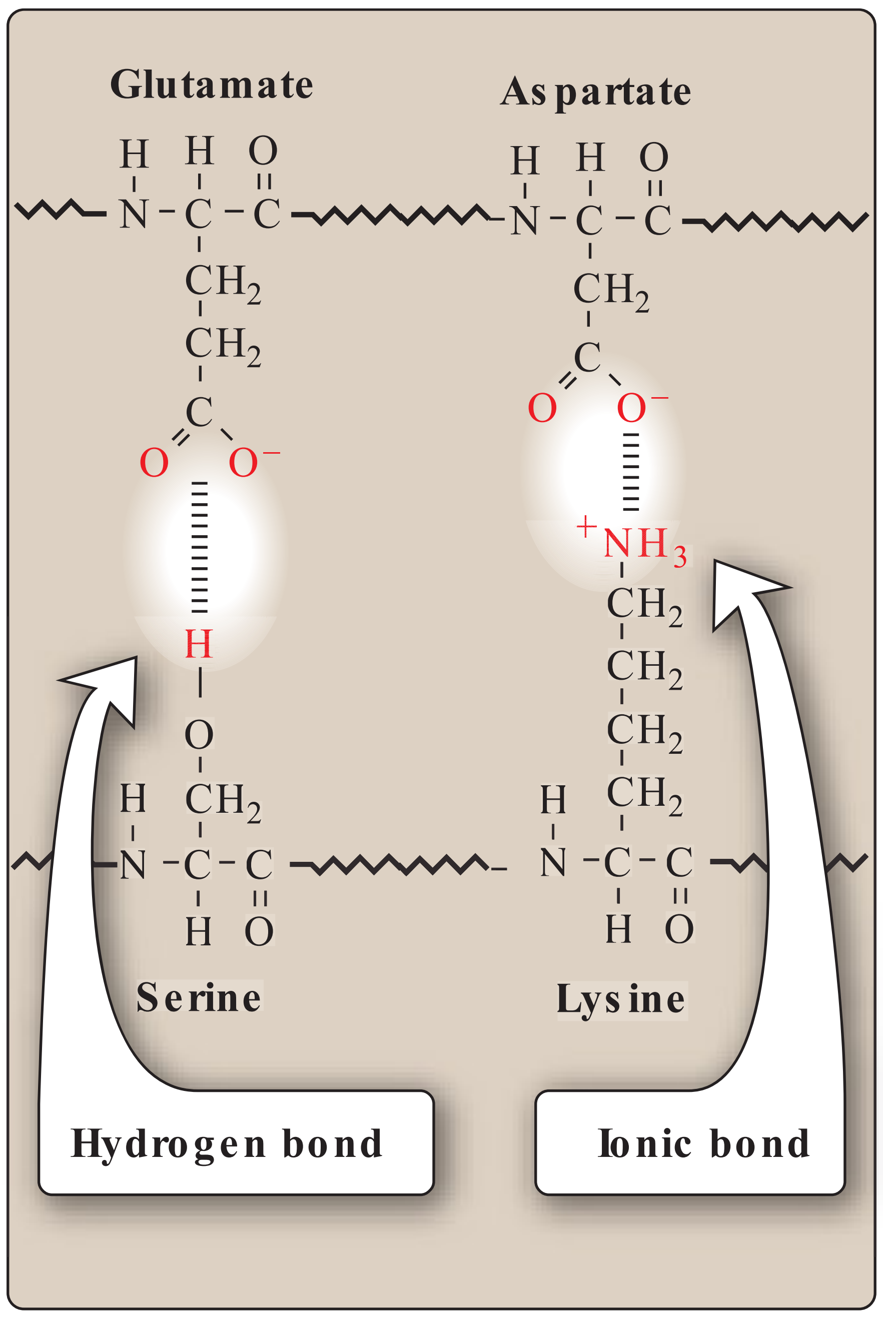


Figure 2.11
Interactions of side chains of amino acids through hydrogen bonds and ionic bonds (salt bridges).

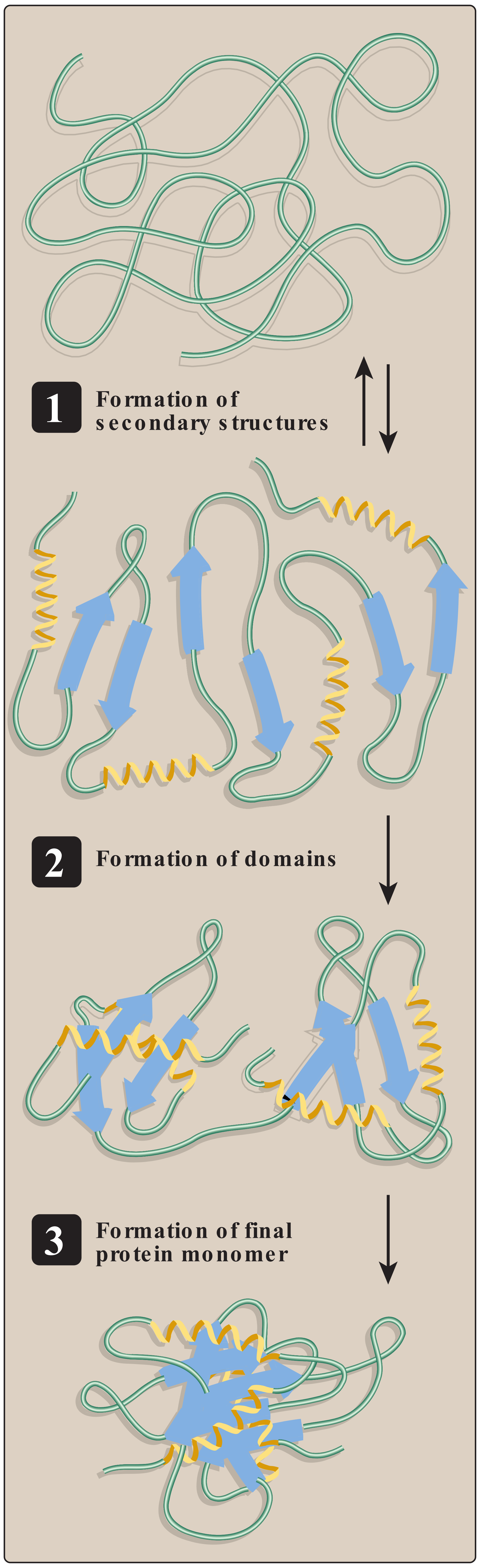

1 Formation of secondary structures
2 Formation of domains
3 Formation of final protein monomer

Figure 2.12
Steps in protein folding (simplified).

C. Protein folding

Interactions between the side chains of amino acids determine how a long polypeptide chain folds into the intricate three-dimensional shape of the functional protein. Protein folding, which occurs within the cell in seconds to minutes, involves nonrandom, ordered pathways. As a peptide folds, secondary structures form driven by the hydrophobic effect (that is, hydrophobic groups come together as water is released). These small structures combine to form larger structures. Additional events stabilize secondary structure and initiate formation of tertiary structure. In the last stage, the peptide achieves its fully folded, native (functional) form characterized by a low-energy state (Figure 2.12). [Note: Some biologically active proteins or segments thereof lack a stable tertiary structure. They are referred to as "intrinsically disordered" proteins.]

D. Denaturation of proteins

Protein denaturation results in the unfolding and disorganization of a protein's secondary and tertiary structures without the hydrolysis of peptide bonds. Denaturing agents include heat, organic solvents, strong acids or bases, detergents, and ions of heavy metals such as lead. Denaturation may, under ideal conditions, be reversible, such that the protein refolds into its original native structure when the denaturing agent is removed. However, most proteins, once denatured, remain permanently disordered. Denatured proteins are often insoluble and precipitate from solution.

E. Role of chaperones in protein folding

The information needed for correct protein folding is contained in the primary structure of the polypeptide. However, most proteins when denatured do not resume their native conformations even under favorable environmental conditions. This is because, for many proteins, folding is a facilitated process that requires a specialized group of proteins, referred to as "molecular chaperones," and adenosine triphosphate hydrolysis. The chaperones, also known as "heat shock proteins" (Hsp), interact with a polypeptide at various stages during the folding process. Some chaperones bind hydrophobic regions of an extended polypeptide and are important in keeping the protein unfolded until its synthesis is completed (for example, Hsp70). Others form cage-like macromolecular structures composed of two stacked rings. The partially folded protein enters the cage, binds the central cavity through hydrophobic interactions, folds, and is released (for example, mitochondrial Hsp60). [Note: Cage-like chaperones are sometimes referred to as "chaperonins."] Chaperones, then, facilitate correct protein folding by binding to and stabilizing exposed, aggregation-prone hydrophobic regions in nascent (and denatured) polypeptides, preventing premature folding.

V. QUATERNARY STRUCTURE OF PROTEINS

Many proteins consist of a single polypeptide chain and are defined as monomeric proteins. However, others may consist of two or more polypeptide chains that may be structurally identical or totally unrelated. The arrangement of these polypeptide subunits is called the quaternary structure of the protein. Subunits are held together primarily by noncovalent interactions (for example, hydrogen bonds, ionic bonds, and hydrophobic interactions). Subunits may either function independently of each other or may work cooperatively, as in hemoglobin, in which the binding of oxygen to one subunit of the tetramer increases the affinity of the other subunits for oxygen (see p. 29).

Isoforms are proteins that perform the same function but have different primary structures. They can arise from different genes or from tissue-specific processing of the product of a single gene. If the proteins function as enzymes, they are referred to as isozymes (see p. 65).

VI. PROTEIN MISFOLDING

Protein folding is a complex process that can sometimes result in improperly folded molecules. These misfolded proteins are usually tagged and degraded within the cell (see p. 444). However, this quality control system is not perfect, and intracellular or extracellular aggregates of misfolded proteins can accumulate, particularly as individuals age. Deposits of misfolded proteins are associated with a number of diseases.

A. Amyloid diseases

Misfolding of proteins may occur spontaneously or be caused by a mutation in a particular gene, which then produces an altered protein. In addition, some apparently normal proteins can, after abnormal proteolytic cleavage, take on a unique conformational state that leads to the formation of long, fibrillar protein assemblies consisting of β-pleated sheets. Accumulation of these insoluble, spontaneously aggregating proteins, called amyloids, has been implicated in degenerative diseases such as Parkinson and Huntington and particularly in the age-related neurodegenerative disorder, Alzheimer disease. The dominant component of the amyloid plaque that accumulates in Alzheimer disease is amyloid β (Aβ), an extracellular peptide containing 40–42 amino acid residues. X-ray crystallography and infrared spectroscopy demonstrate a characteristic β-pleated sheet conformation in nonbranching fibrils. This peptide, when aggregated in a β-pleated sheet configuration, is neurotoxic and is the central pathogenic event leading to the cognitive impairment characteristic of the disease. The Aβ that is deposited in the brain in Alzheimer disease is derived by enzymic cleavages (by secretases) from the larger amyloid precursor protein, a single transmembrane protein expressed on the cell surface in the brain and other tissues (Figure 2.13). The Aβ peptides aggregate, generating the amyloid that is found in the brain parenchyma and around blood vessels. Most cases of Alzheimer disease are not genetically based, although at least 5% of cases are familial. A second biologic factor involved in the development of Alzheimer disease is the accumulation of neurofibrillary tangles inside neurons. A key component of these tangled fibers is an abnormal form (hyperphosphorylated and insoluble) of the tau (τ) protein, which, in its healthy version, helps in the assembly of the microtubular structure. The defective τ appears to block the actions of its normal counterpart.

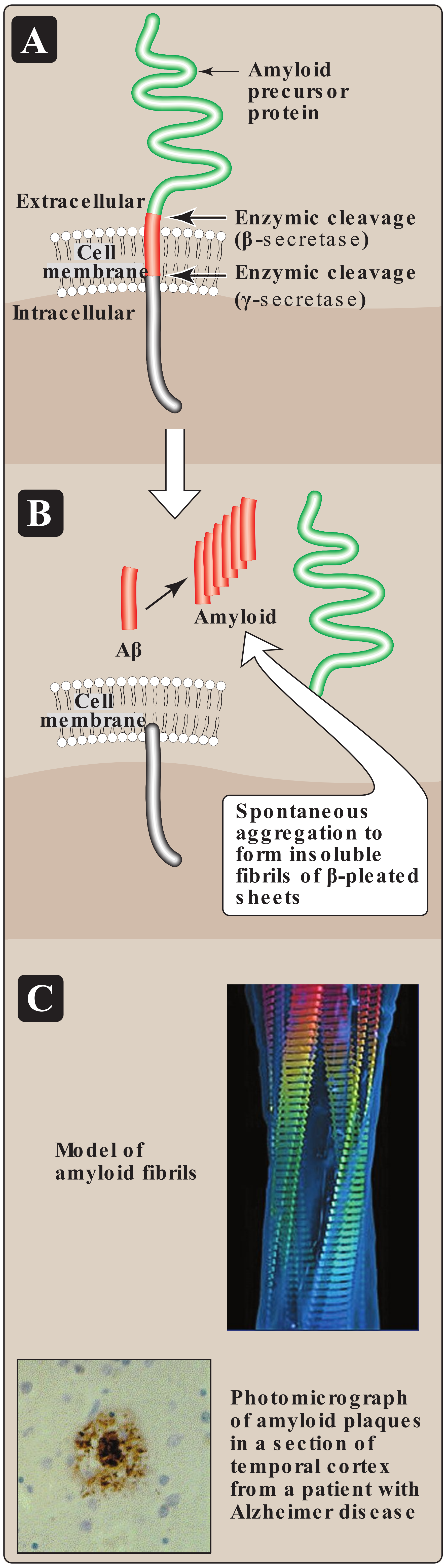
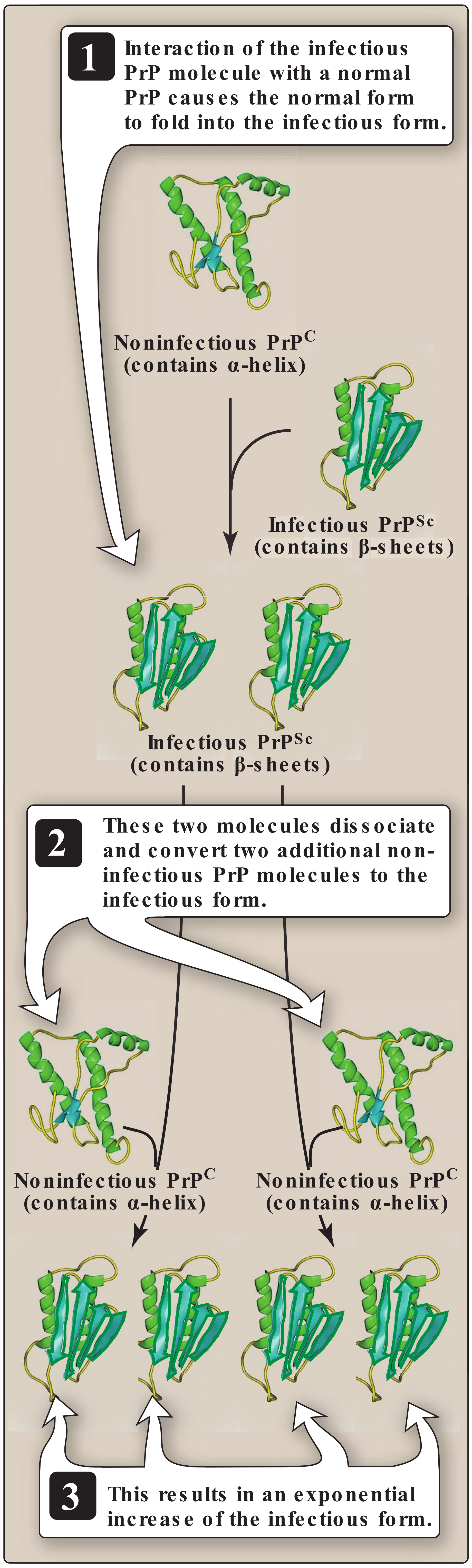

A
Amyloid precursor protein
Extracellular
Cell membrane
Intracellular
Enzymic cleavage (β-secretase)
Enzymic cleavage (γ-secretase)
B
Aβ
Amyloid
Cell membrane
Spontaneous aggregation to form insoluble fibrils of β-pleated sheets
C
Model of amyloid fibrils
Photomicrograph of amyloid plaques in a section of temporal cortex from a patient with Alzheimer disease

Figure 2.13
Formation of amyloid plaques found in Alzheimer disease (AD). [Note: Mutations to presenilin, the catalytic subunit of γ-secretase, are the most common cause of familial AD.]

B. Prion diseases

The prion protein (PrP) has been strongly implicated as the causative agent of transmissible spongiform encephalopathies (TSEs), including Creutzfeldt-Jakob disease in humans, scrapie in sheep, and bovine spongiform encephalopathy in cattle (popularly called "mad cow" disease). After an extensive series of purification procedures, scientists were surprised to find that the infectivity of the agent causing scrapie in sheep was associated with a single protein species that was not complexed with detectable nucleic acid. This infectious protein is designated PrP^{Sc} (Sc = scrapie). It is highly resistant to proteolytic degradation and tends to form insoluble aggregates of fibrils, similar to the amyloid found in some other diseases of the brain. A noninfectious form of PrP^{C} (C = cellular), encoded by the same gene as the infectious agent, is present in normal mammalian brains on the surface of neurons and glial cells. Thus, PrP^{C} is a host protein. No primary structure differences or alternate posttranslational modifications have been found between the normal and the infectious forms of the protein. The key to becoming infectious apparently lies in changes in the three-dimensional conformation of PrP^{C}. It has been observed that a number of α-helices present in noninfectious PrP^{C} are replaced by β-sheets in the infectious form (Figure 2.14). It is presumably this conformational difference that confers relative resistance to proteolytic degradation of infectious prions and permits them to be distinguished from the normal PrP^{C} in infected tissue. The infective agent is, thus, an altered version of a normal protein, which acts as a "template" for converting the normal protein to the pathogenic conformation. The TSEs are invariably fatal, and no treatment is currently available that can alter this outcome.



1 Interaction of the infectious PrP molecule with a normal PrP causes the normal form to fold into the infectious form.

2 These two molecules dissociate and convert two additional noninfectious PrP molecules to the infectious form.

3 This results in an exponential increase of the infectious form.

Noninfectious PrP^{C} (contains α-helix)
Infectious PrP^{Sc} (contains β-sheets)
Infectious PrP^{Sc} (contains β-sheets)
Noninfectious PrP^{C} (contains α-helix)
Noninfectious PrP^{C} (contains α-helix)

Figure 2.14
One proposed mechanism for multiplication of infectious prion agents. PrP = prion protein; PrP^{c} = prion protein cellular; PrP^{Sc} = prion protein scrapie.

VII. CHAPTER SUMMARY

Central to understanding protein structure is the concept of the **native conformation** (Figure 2.15), which is the functional, fully folded protein structure (for example, an active enzyme or structural protein). The unique three-dimensional structure of the native conformation is determined by its **primary structure**, that is, its amino acid sequence. Interactions between the amino acid side chains guide the folding of the polypeptide chain to form **secondary**, **tertiary**, and (sometimes) **quaternary** structures, which cooperate in stabilizing the native conformation of the protein. In addition, a specialized group of proteins named **chaperones** is required for the proper folding of many species of proteins. **Protein denaturation** results in the unfolding and disorganization of the protein's structure, which are not accompanied by hydrolysis of peptide bonds. Denaturation may be reversible or, more commonly, irreversible. Disease can occur when an apparently normal protein assumes a conformation that is cytotoxic, as in the case of Alzheimer disease and the **transmissible spongiform encephalopathies** (**TSEs**), including **Creutzfeldt-Jakob disease**. In **Alzheimer disease,** normal proteins, after abnormal chemical processing, take on a unique conformational state that leads to the formation of neurotoxic **amyloid β peptide** (**Aβ**) assemblies consisting of β-pleated sheets. In TSEs, the infective agent is an altered version of a normal **prion protein** that acts as a "template" for converting normal protein to the pathogenic conformation.

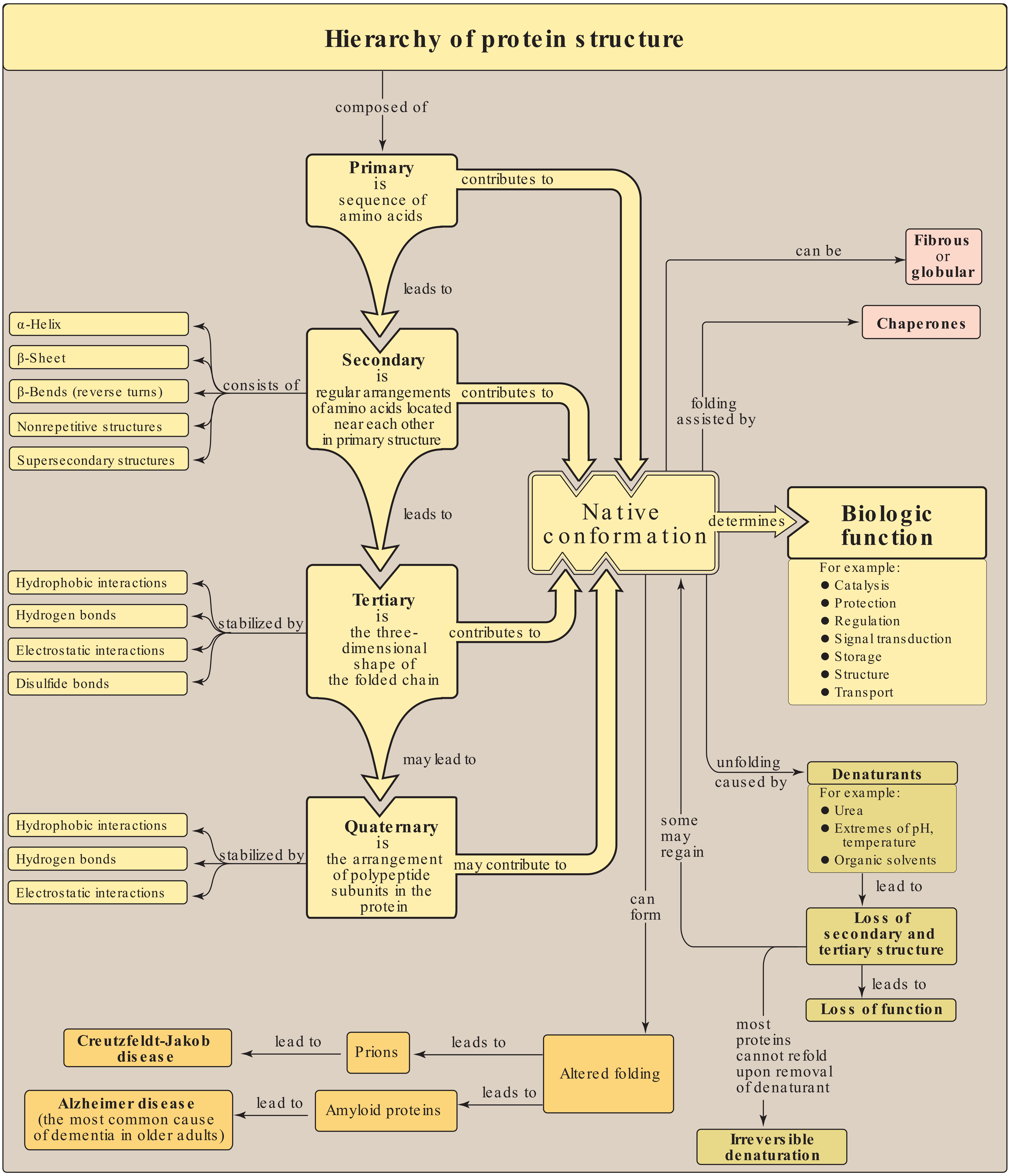

Hierarchy of protein structure
composed of
Primary is sequence of amino acids
contributes to
leads to
α-Helix
β-Sheet
β-Bends (reverse turns)
Nonrepetitive structures
Supersecondary structures
consists of
Secondary is regular arrangements of amino acids located near each other in primary structure
contributes to
leads to
Hydrophobic interactions
Hydrogen bonds
Electrostatic interactions
Disulfide bonds
stabilized by
Tertiary is the three-dimensional shape of the folded chain
contributes to
may lead to
Hydrophobic interactions
Hydrogen bonds
Electrostatic interactions
stabilized by
Quaternary is the arrangement of polypeptide subunits in the protein
may contribute to
Native conformation
can be
Fibrous or globular
folding assisted by
Chaperones
determines
Biologic function
For example:
● Catalysis
● Protection
● Regulation
● Signal transduction
● Storage
● Structure
● Transport
unfolding caused by
Denaturants
For example:
● Urea
● Extremes of pH, temperature
● Organic solvents
lead to
Loss of secondary and tertiary structure
leads to
Loss of function
some may regain
most proteins cannot refold upon removal of denaturant
Irreversible denaturation
can form
Altered folding
leads to
Prions
lead to
Creutzfeldt-Jakob disease
leads to
Amyloid proteins
lead to
Alzheimer disease (the most common cause of dementia in older adults)

Figure 2.15
Key concept map for protein structure.

Globular Proteins

3

I. OVERVIEW

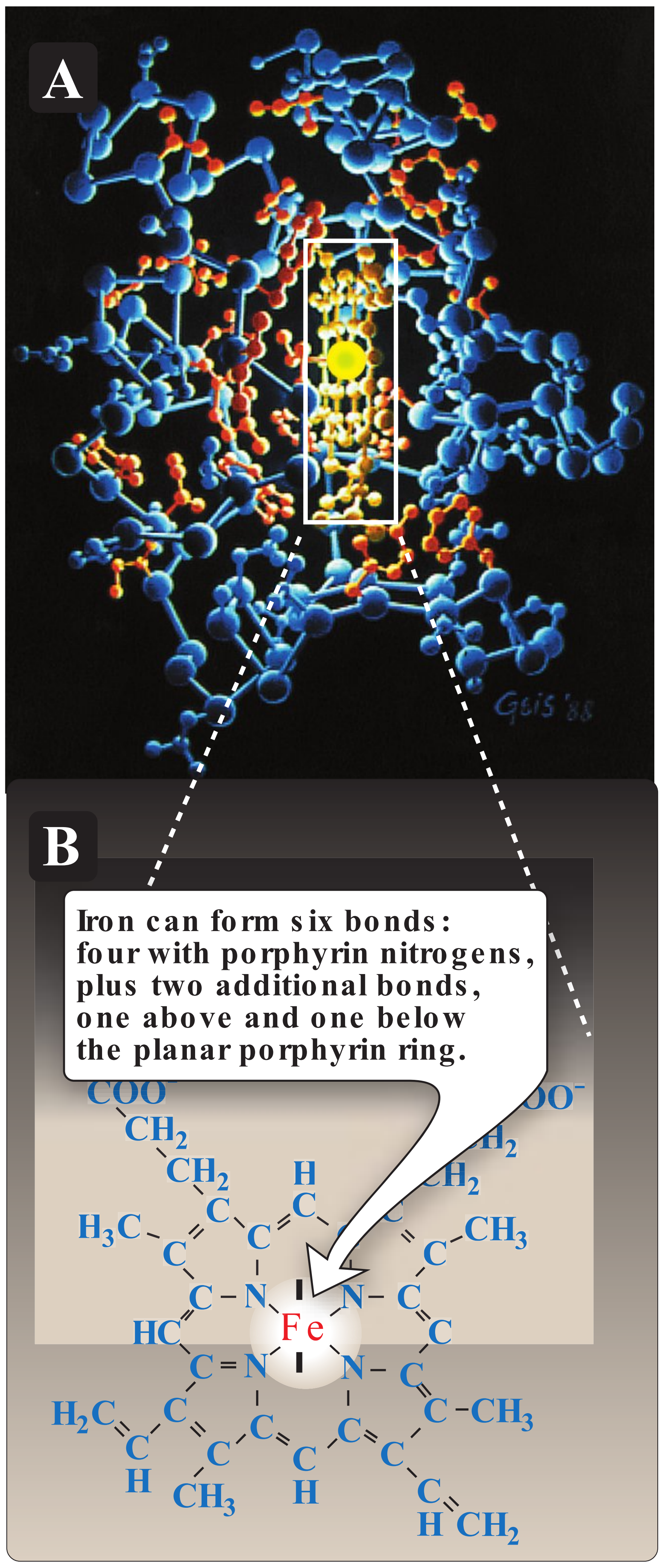
The previous chapter described the types of secondary and tertiary structures that are the bricks and mortar of protein architecture. By arranging these fundamental structural elements in different combinations, widely diverse proteins can be constructed that are capable of various specialized functions. This chapter examines the relationship between structure and function for the clinically important globular hemeproteins. Fibrous structural proteins are discussed in Chapter 4.

II. GLOBULAR HEMEPROTEINS

Hemeproteins are a group of specialized proteins that contain heme as a tightly bound prosthetic group. (See p. 54 for a discussion of prosthetic groups.) The role of the heme group is dictated by the environment created by the three-dimensional structure of the protein. For example, the heme group of a cytochrome functions as an electron carrier that is alternately oxidized and reduced (see p. 76). In contrast, the heme group of the enzyme catalase is part of the active site of the enzyme that catalyzes the breakdown of hydrogen peroxide (see p. 148). In hemoglobin and myoglobin, the two most abundant hemeproteins in humans, the heme group serves to reversibly bind oxygen.

A. Structure of heme

Heme is a complex of protoporphyrin IX and ferrous iron (Fe^{2+}) (Figure 3.1). The iron is held in the center of the heme molecule by bonds to the four nitrogens of the porphyrin ring. The heme Fe^{2+} can form two additional bonds, one on each side of the planar porphyrin ring. In myoglobin and hemoglobin, one of these positions is coordinated to the side chain of a histidine residue of the globin molecule, whereas the other position is available to bind oxygen (Figure 3.2). (See pp. 278 and 282 for a discussion of the synthesis and degradation of heme.)



Iron can form six bonds: four with porphyrin nitrogens, plus two additional bonds, one above and one below the planar porphyrin ring.

Figure 3.1
A. Hemeprotein (cytochrome c).
B. Structure of heme.

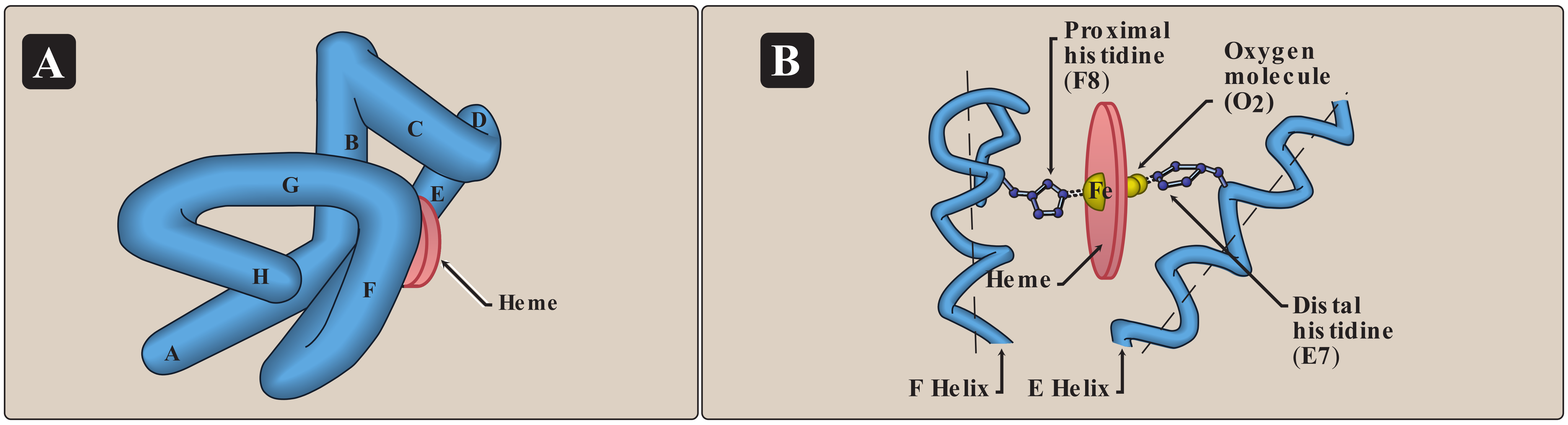


Figure 3.2
A. Model of myoglobin showing helices A to H. B. Schematic diagram of the oxygen-binding site of myoglobin.

B. Structure and function of myoglobin

Myoglobin, a hemeprotein present in heart and skeletal muscle, functions both as a reservoir for oxygen and as an oxygen carrier that increases the rate of transport of oxygen within the muscle cell. [Note: Mouse myoglobin double knockouts (see p. 486) have, surprisingly, an apparently normal phenotype.] Myoglobin consists of a single polypeptide chain that is structurally similar to the individual polypeptide chains of the tetrameric hemoglobin molecule. This homology makes myoglobin a useful model for interpreting some of the more complex properties of hemoglobin.

1. **α-Helical content:** Myoglobin is a compact molecule, with approximately 80% of its polypeptide chain folded into eight stretches of α-helix. These α-helical regions, labeled A to H in Figure 3.2A, are terminated either by the presence of proline, whose five-membered ring cannot be accommodated in an α-helix (see p. 16) or by β-bends and loops stabilized by hydrogen bonds and ionic bonds (see p. 17). [Note: Ionic bonds are also termed electrostatic interactions or salt bridges.]

2. **Location of polar and nonpolar amino acid residues:** The interior of the myoglobin molecule is composed almost entirely of nonpolar amino acids. They are packed closely together, forming a structure stabilized by hydrophobic interactions between these clustered residues (see p. 19). In contrast, polar amino acids are located almost exclusively on the surface, where they can form hydrogen bonds, both with each other and with water.

3. **Binding of the heme group:** The heme group of the myoglobin molecule sits in a crevice, which is lined with nonpolar amino acids. Notable exceptions are two histidine residues (Figure 3.2B). One, the proximal histidine (F8), binds directly to the iron of heme. The second, or distal histidine (E7), does not directly interact with the heme group but helps stabilize the binding of oxygen to the ferrous iron. The protein, or globin, portion of myoglobin thus creates a special microenvironment for the heme that permits the reversible binding of one oxygen molecule (oxygenation). The simultaneous loss of electrons by the ferrous iron (oxidation to the ferric form) occurs only rarely.

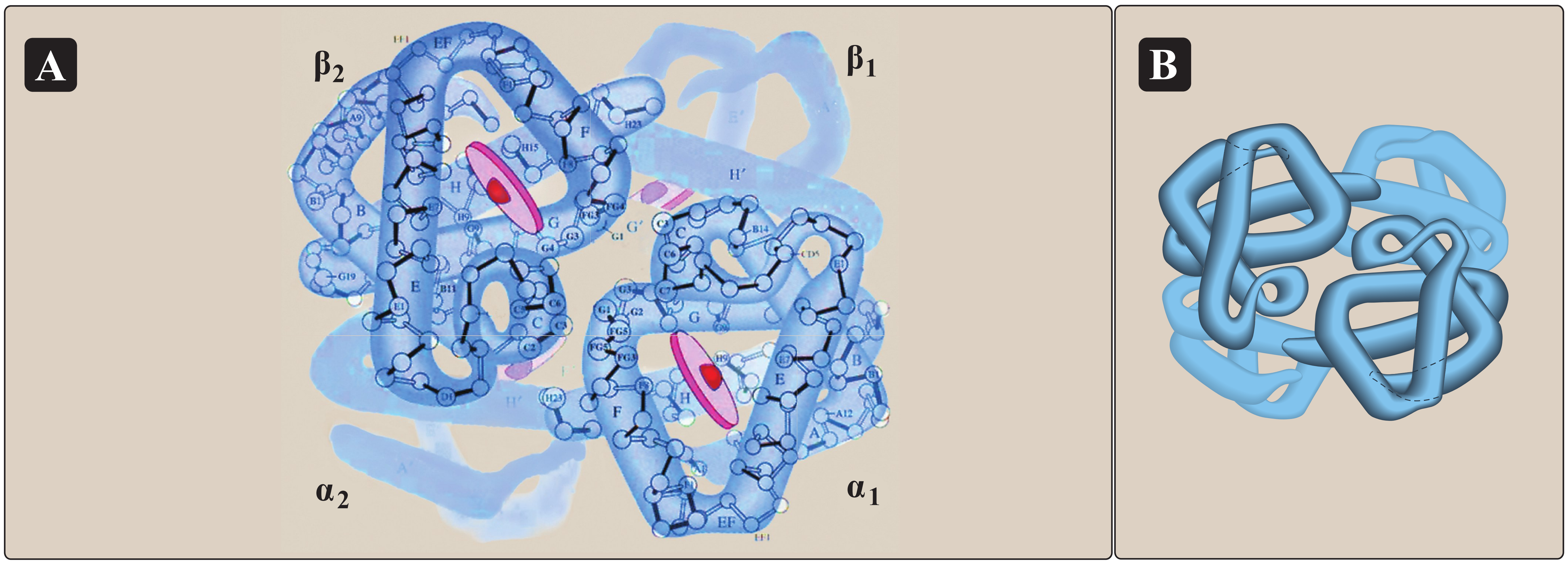


Figure 3.3
A. Structure of hemoglobin showing the polypeptide backbone. B. Simplified drawing showing the helices.

C. Structure and function of hemoglobin

Hemoglobin is found exclusively in red blood cells (RBC), where its main function is to transport oxygen (O_2) from the lungs to the capillaries of the tissues. Hemoglobin A, the major hemoglobin in adults, is composed of four polypeptide chains (two α chains and two β chains) held together by noncovalent interactions (Figure 3.3). Each chain (subunit) has stretches of α-helical structure and a hydrophobic heme-binding pocket similar to that described for myoglobin. However, the tetrameric hemoglobin molecule is structurally and functionally more complex than myoglobin. For example, hemoglobin can transport H^+ and CO_2 from the tissues to the lungs and can carry four molecules of O_2 from the lungs to the cells of the body. Furthermore, the oxygen-binding properties of hemoglobin are regulated by interaction with allosteric effectors (see p. 29).

Obtaining O_2 from the atmosphere solely by diffusion greatly limits the size of organisms. Circulatory systems overcome this, but transport molecules such as hemoglobin are also required because O_2 is only slightly soluble in aqueous solutions such as blood.

1. **Quaternary structure of hemoglobin:** The hemoglobin tetramer can be envisioned as being composed of two identical dimers, $(\alpha\beta)_1$ and $(\alpha\beta)_2$. The two polypeptide chains within each dimer are held tightly together primarily by hydrophobic interactions (Figure 3.4). [Note: In this instance, hydrophobic amino acid residues are localized not only in the interior of the molecule, but also in a region on the surface of each subunit. Multiple interchain hydrophobic interactions form strong associations between α-subunits and β-subunits in the dimers.] In contrast, the two dimers are

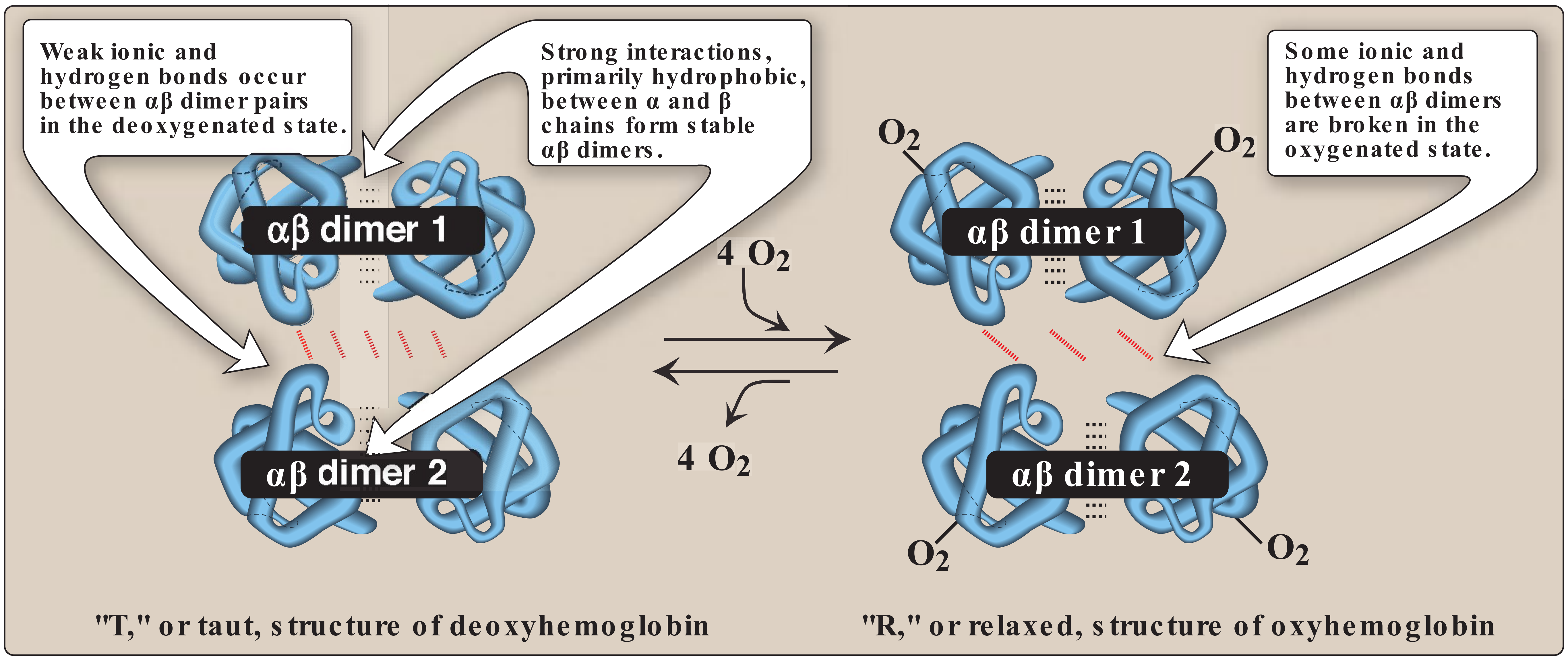

Weak ionic and hydrogen bonds occur between αβ dimer pairs in the deoxygenated state.
Strong interactions, primarily hydrophobic, between α and β chains form stable αβ dimers.
Some ionic and hydrogen bonds between αβ dimers are broken in the oxygenated state.
αβ dimer 1
αβ dimer 2
αβ dimer 1
αβ dimer 2
4 O2
4 O2
O2
O2
O2
O2
"T," or taut, structure of deoxyhemoglobin
"R," or relaxed, structure of oxyhemoglobin

Figure 3.4
Schematic diagram showing structural changes resulting from oxygenation and deoxygenation of hemoglobin.

held together primarily by polar bonds. The weaker interactions between the dimers allows them to move with respect to one other. This movement results in the two dimers occupying different relative positions in deoxyhemoglobin as compared with oxyhemoglobin (see Figure 3.4). [Note: The binding of O_2 to the heme iron pulls the iron into the plane of the heme. Because the iron is also linked to the proximal histidine (F8), there is movement of the globin chains that alters the interface between the αβ dimers.]

a. **T form:** The deoxy form of hemoglobin is called the "T," or taut (tense) form. In the T form, the two αβ dimers interact through a network of ionic bonds and hydrogen bonds that constrain the movement of the polypeptide chains. The T conformation is the low-oxygen-affinity form of hemoglobin.

b. **R form:** The binding of O_2 to hemoglobin causes the rupture of some of the polar bonds between the αβ dimers, allowing movement. This leads to a structure called the "R," or relaxed form (see Figure 3.4). The R conformation is the high-oxygen-affinity form of hemoglobin.

D. Binding of oxygen to myoglobin and hemoglobin

Myoglobin can bind only one molecule of O_2, because it contains only one heme group. In contrast, hemoglobin can bind four O_2 molecules, one at each of its four heme groups. The degree of saturation (Y) of these oxygen-binding sites on all myoglobin or hemoglobin molecules can vary between zero (all sites are empty) and 100% (all sites are full), as shown in Figure 3.5. [Note: Pulse oximetry is a noninvasive, indirect method of measuring the O_2 saturation of arterial blood based on differences in light absorption by oxyhemoglobin and deoxyhemoglobin.]

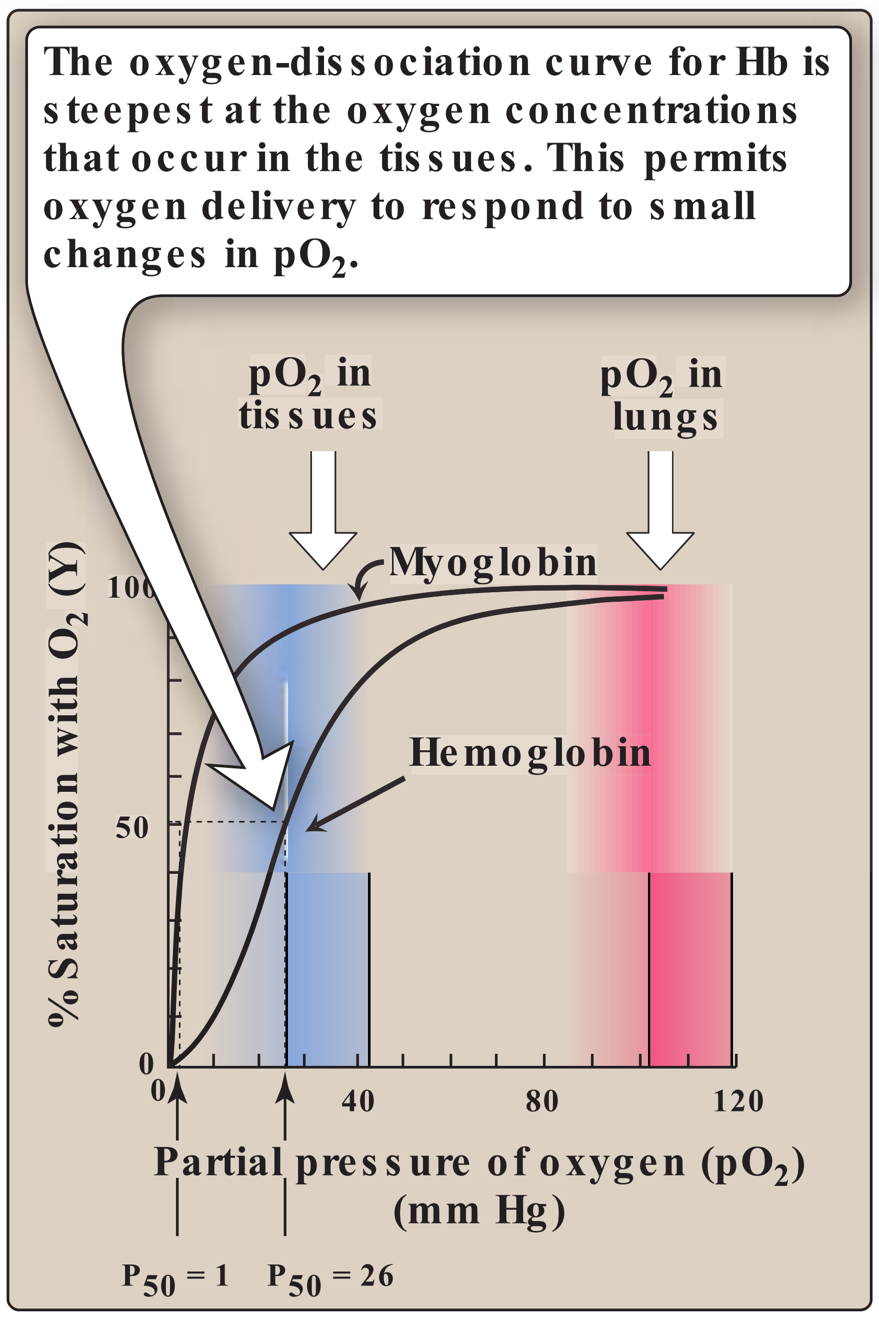
1. **Oxygen-dissociation curve:** A plot of Y measured at different partial pressures of oxygen (pO_2) is called the oxygen-dissociation curve. [Note: pO_2 may also be represented as PO_2.] The curves for myoglobin and hemoglobin show important differences (see Figure 3.5). This graph illustrates that myoglobin has a higher oxygen affinity at all pO_2 values than does hemoglobin. The partial pressure of oxygen needed to achieve half-saturation of the binding sites (P_{50}) is approximately 1 mm Hg for myoglobin and 26 mm Hg for hemoglobin. The higher the oxygen affinity (that is, the more tightly oxygen binds), the lower the P_{50}.

   a. **Myoglobin:** The oxygen-dissociation curve for myoglobin has a hyperbolic shape (see Figure 3.5). This reflects the fact that myoglobin reversibly binds a single molecule of oxygen. Thus, oxygenated (MbO_2) and deoxygenated (Mb) myoglobin exist in a simple equilibrium:

$$Mb + O_2 \rightleftarrows MbO_2$$

   The equilibrium is shifted to the right or to the left as oxygen is added to or removed from the system. [Note: Myoglobin is designed to bind oxygen released by hemoglobin at the low pO_2 found in muscle. Myoglobin, in turn, releases oxygen within the muscle cell in response to oxygen demand.]

   b. **Hemoglobin:** The oxygen-dissociation curve for hemoglobin is sigmoidal in shape (see Figure 3.5), indicating that the subunits cooperate in binding oxygen. Cooperative binding of oxygen by the four subunits of hemoglobin means that the binding of an oxygen molecule at one heme group increases the oxygen affinity of the remaining heme groups in the same hemoglobin tetramer (Figure 3.6). This effect is referred to as heme–heme interaction (see below). Although it is more difficult for the first oxygen molecule to bind to hemoglobin, the subsequent binding of oxygen occurs with high affinity, as shown by the steep upward curve in the region near 20–30 mm Hg (see Figure 3.5).



The oxygen-dissociation curve for Hb is steepest at the oxygen concentrations that occur in the tissues. This permits oxygen delivery to respond to small changes in pO_2.

Figure 3.5
Oxygen-dissociation curves for myoglobin and hemoglobin (Hb).

E. Allosteric effects

The ability of hemoglobin to reversibly bind oxygen is affected by the pO_2 (through heme–heme interactions as described above), the pH of the environment, the partial pressure of carbon dioxide (pCO_2) and the availability of 2,3-bisphosphoglycerate. These are collectively called allosteric ("other site") effectors, because their interaction at one site on the hemoglobin molecule affects the binding of oxygen to heme groups at other sites on the molecule. [Note: The binding of oxygen to monomeric myoglobin is not influenced by allosteric effectors.]

1. **Heme–heme interactions:** The sigmoidal oxygen-dissociation curve reflects specific structural changes that are initiated at one heme group and transmitted to other heme groups in the hemoglobin tetramer. The net effect is that the affinity of hemoglobin for the last oxygen bound is approximately 300 times greater than its affinity for the first oxygen bound.

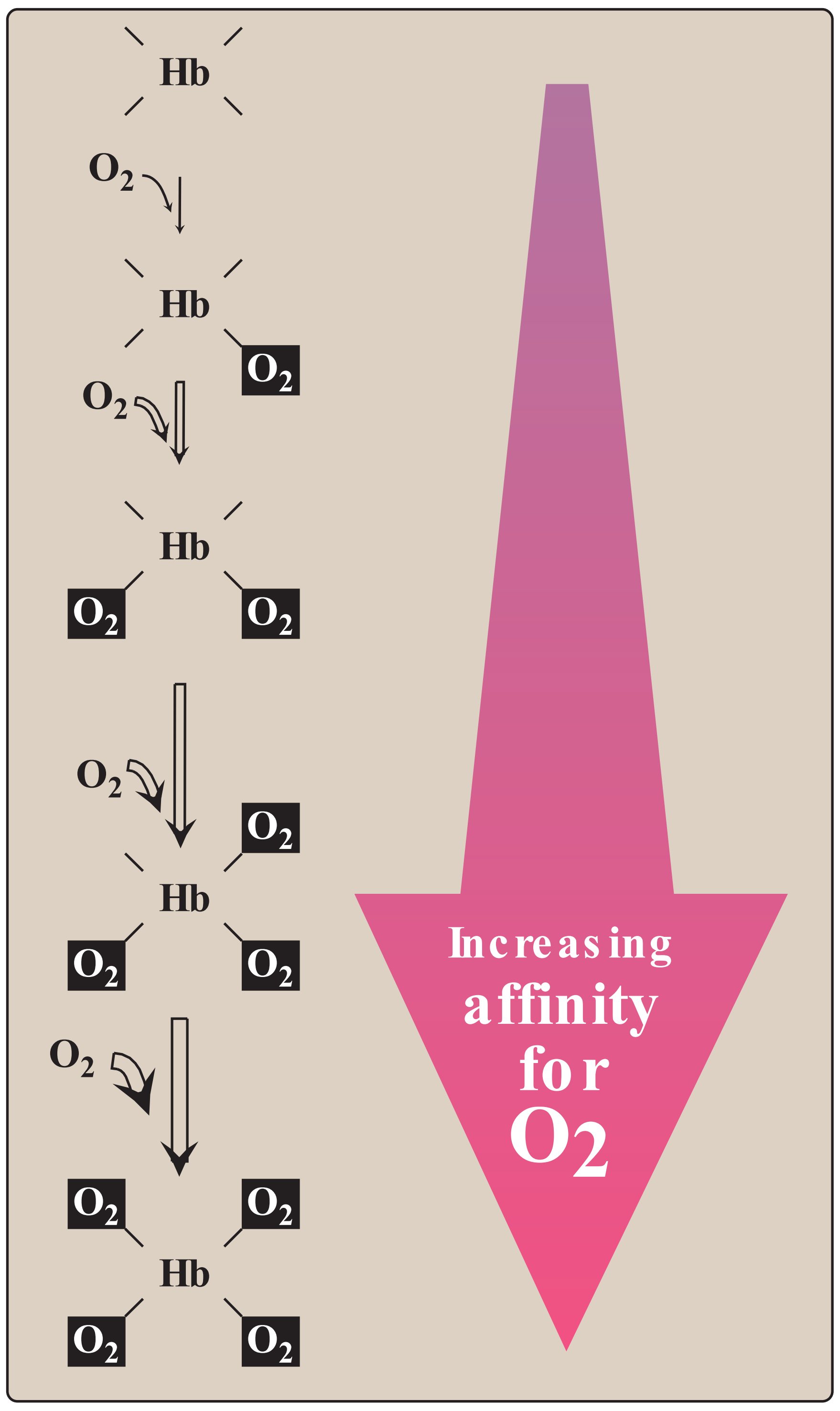


Figure 3.6
Hemoglobin (Hb) binds successive molecules of oxygen with increasing affinity.

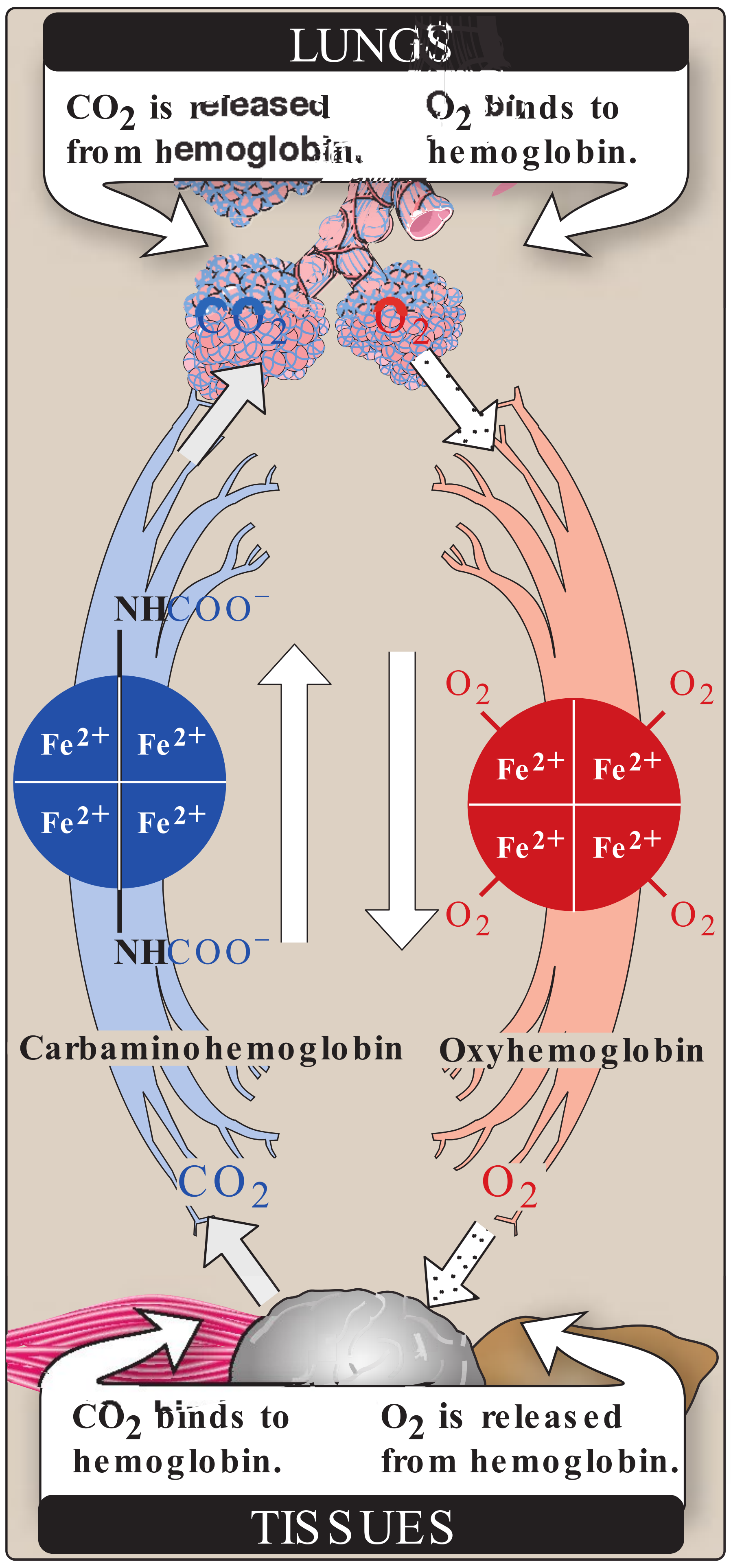


Figure 3.7
Transport of oxygen and carbon dioxide by hemoglobin. Fe = iron.

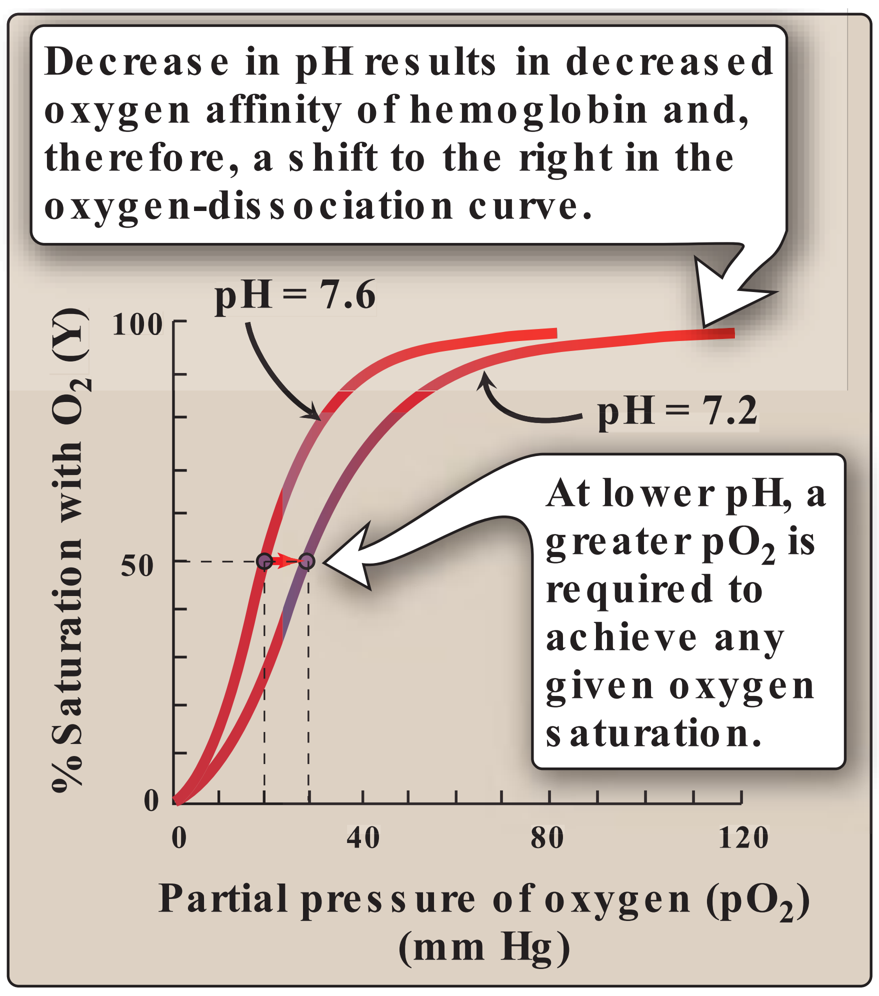


Figure 3.8
Effect of pH on the oxygen affinity of hemoglobin. Protons are allosteric effectors of hemoglobin.

a. **Loading and unloading oxygen:** The cooperative binding of oxygen allows hemoglobin to deliver more oxygen to the tissues in response to relatively small changes in the partial pressure of oxygen. This can be seen in Figure 3.5, which indicates pO_2 in the alveoli of the lung and the capillaries of the tissues. For example, in the lung, the concentration of oxygen is high, and hemoglobin becomes virtually saturated (or "loaded") with oxygen. In contrast, in the peripheral tissues, oxyhemoglobin releases (or "unloads") much of its oxygen for use in the oxidative metabolism of the tissues (Figure 3.7).

b. **Significance of the sigmoidal oxygen-dissociation curve:** The steep slope of the oxygen-dissociation curve over the range of oxygen concentrations that occur between the lungs and the tissues permits hemoglobin to carry and deliver oxygen efficiently from sites of high to sites of low pO_2. A molecule with a hyperbolic oxygen-dissociation curve, such as myoglobin, could not achieve the same degree of oxygen release within this range of partial pressures of oxygen. Instead, it would have maximum affinity for oxygen throughout this oxygen pressure range and, therefore, would deliver no oxygen to the tissues.

2. **Bohr effect:** The release of oxygen from hemoglobin is enhanced when the pH is lowered or when the hemoglobin is in the presence of an increased pCO_2. Both result in a decreased oxygen affinity of hemoglobin and, therefore, a shift to the right in the oxygen-dissociation curve (Figure 3.8), and both, then, stabilize the T (deoxy) form. This change in oxygen binding is called the Bohr effect. Conversely, raising the pH or lowering the concentration of CO_2 results in a greater affinity for oxygen, a shift to the left in the oxygen-dissociation curve, and stabilization of the R (oxy) form.

a. **Source of the protons that lower the pH:** The concentration of both H^+ and CO_2 in the capillaries of metabolically active tissues is higher than that observed in alveolar capillaries of the lungs, where CO_2 is released into the expired air. In the tissues, CO_2 is converted by carbonic anhydrase to carbonic acid:

$$CO_2 + H_2O \quad \rightleftarrows \quad H_2CO_3$$

which spontaneously loses a proton, becoming bicarbonate (the major blood buffer):

$$H_2CO_3 \quad \rightleftarrows \quad HCO_3^- + H^+$$

The H^+ produced by this pair of reactions contributes to the lowering of pH. This differential pH gradient (that is, lungs having a higher pH and tissues a lower pH) favors the unloading of oxygen in the peripheral tissues and the loading of oxygen in the lung. Thus, the oxygen affinity of the hemoglobin molecule responds to small shifts in pH between the lungs and oxygen-consuming tissues, making hemoglobin a more efficient transporter of oxygen.

b. Mechanism of the Bohr effect: The Bohr effect reflects the fact that the deoxy form of hemoglobin has a greater affinity for protons than does oxyhemoglobin. This effect is caused by ionizable groups such as specific histidine side chains that have a higher pK_a in deoxyhemoglobin than in oxyhemoglobin. Therefore, an increase in the concentration of protons (resulting in a decrease in pH) causes these groups to become protonated (charged) and able to form ionic bonds (salt bridges). These bonds preferentially stabilize the deoxy form of hemoglobin, producing a decrease in oxygen affinity. [Note: Hemoglobin, then, is an important blood buffer.]

The Bohr effect can be represented schematically as:

$$\underset{\text{oxyhemoglobin}}{HbO_2 + H^+} \rightleftarrows \underset{\text{deoxyhemoglobin}}{HbH + O_2}$$

where an increase in protons (or a lower pO_2) shifts the equilibrium to the right (favoring deoxyhemoglobin), whereas an increase in pO_2 (or a decrease in protons) shifts the equilibrium to the left.

3. Effect of 2,3-bisphosphoglycerate on oxygen affinity: 2,3-Bisphosphoglycerate (2,3-BPG) is an important regulator of the binding of oxygen to hemoglobin. It is the most abundant organic phosphate in the RBC, where its concentration is approximately that of hemoglobin. 2,3-BPG is synthesized from an intermediate of the glycolytic pathway (Figure 3.9; see p. 101 for a discussion of 2,3-BPG synthesis in glycolysis).

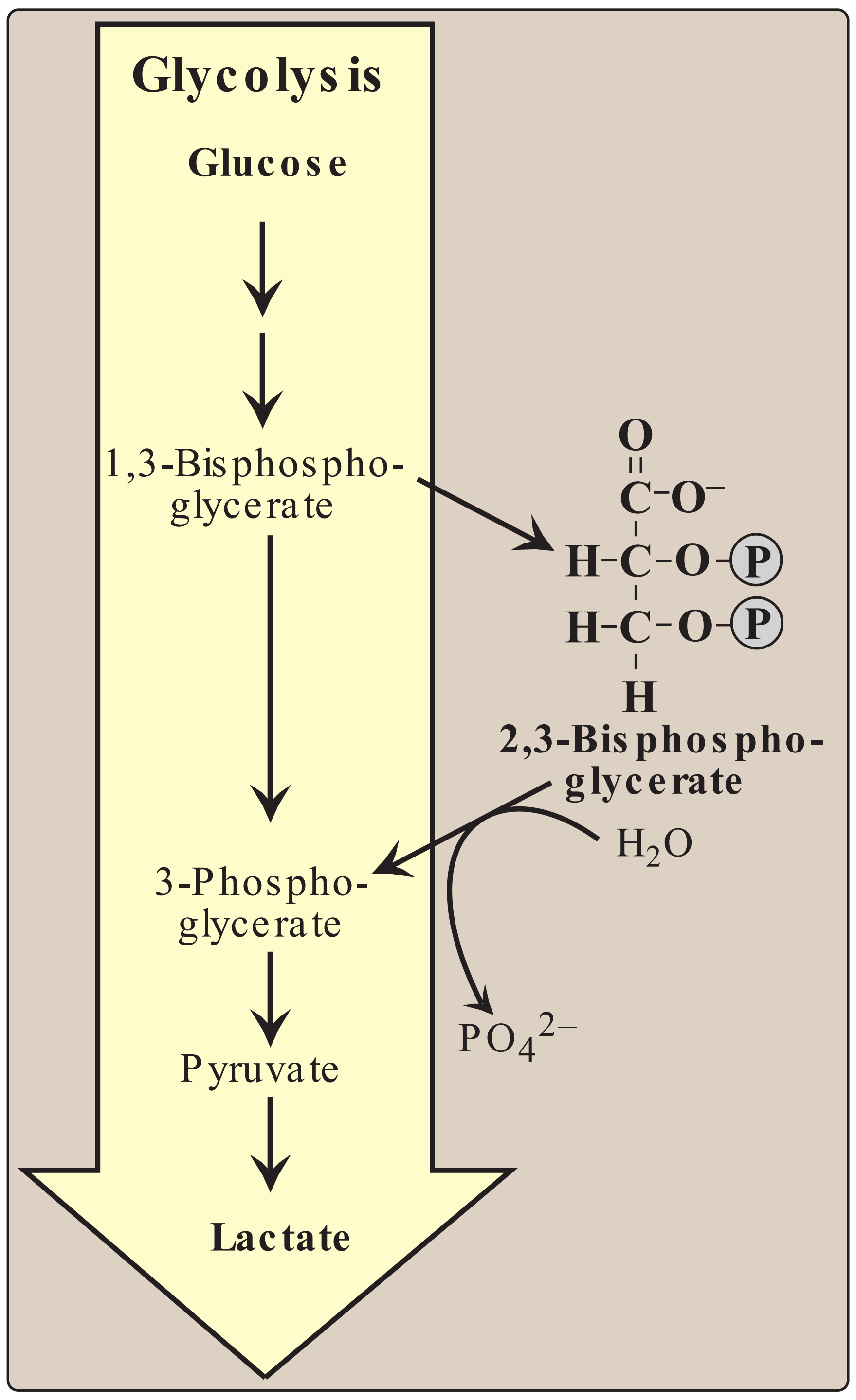


Figure 3.9
Synthesis of 2,3-bisphosphoglycerate. [Note: Ⓟ is a phosphoryl group, PO_3^{2-}.] In older literature, 2,3-bisphosphoglycerate (2,3-BPG) may be referred to as 2,3-diphosphoglycerate (2,3-DPG).

a. Binding of 2,3-BPG to deoxyhemoglobin: 2,3-BPG decreases the O_2 affinity of hemoglobin by binding to deoxyhemoglobin but not to oxyhemoglobin. This preferential binding stabilizes the T conformation of deoxyhemoglobin. The effect of binding 2,3-BPG can be represented schematically as:

$$\underset{\text{oxyhemoglobin}}{HbO_2 + \text{2,3-BPG}} \rightleftarrows \underset{\text{deoxyhemoglobin}}{\text{Hb–2,3-BPG} + O_2}$$

b. Binding site of 2,3-BPG: One molecule of 2,3-BPG binds to a pocket, formed by the two β-globin chains, in the center of the deoxyhemoglobin tetramer (Figure 3.10). This pocket contains several positively charged amino acids that form ionic bonds with the negatively charged phosphate groups of 2,3-BPG. [Note: Replacement of one of these amino acids can result in hemoglobin variants with abnormally high oxygen affinity that may be compensated for by increased RBC production (erythrocytosis).] 2,3-BPG is expelled with oxygenation of the hemoglobin.

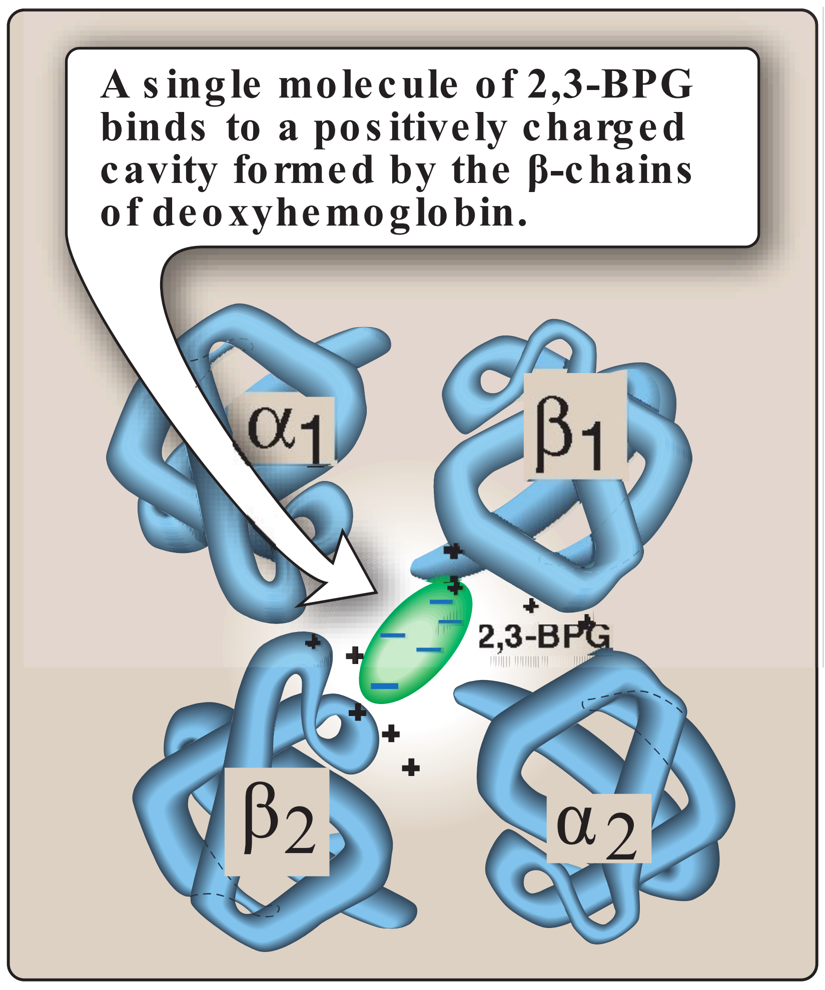

A single molecule of 2,3-BPG binds to a positively charged cavity formed by the β-chains of deoxyhemoglobin.

Figure 3.10
Binding of 2,3-bisphosphoglycerate (2,3-BPG) by deoxyhemoglobin.

c. Shift of the oxygen-dissociation curve: Hemoglobin from which 2,3-BPG has been removed has a high affinity for oxygen. However, as seen in the RBC, the presence of 2,3-BPG significantly reduces the affinity of hemoglobin for oxygen, shifting the oxygen-dissociation curve to the right (Figure 3.11). This reduced affinity enables hemoglobin to release oxygen efficiently at the partial pressures found in the tissues.

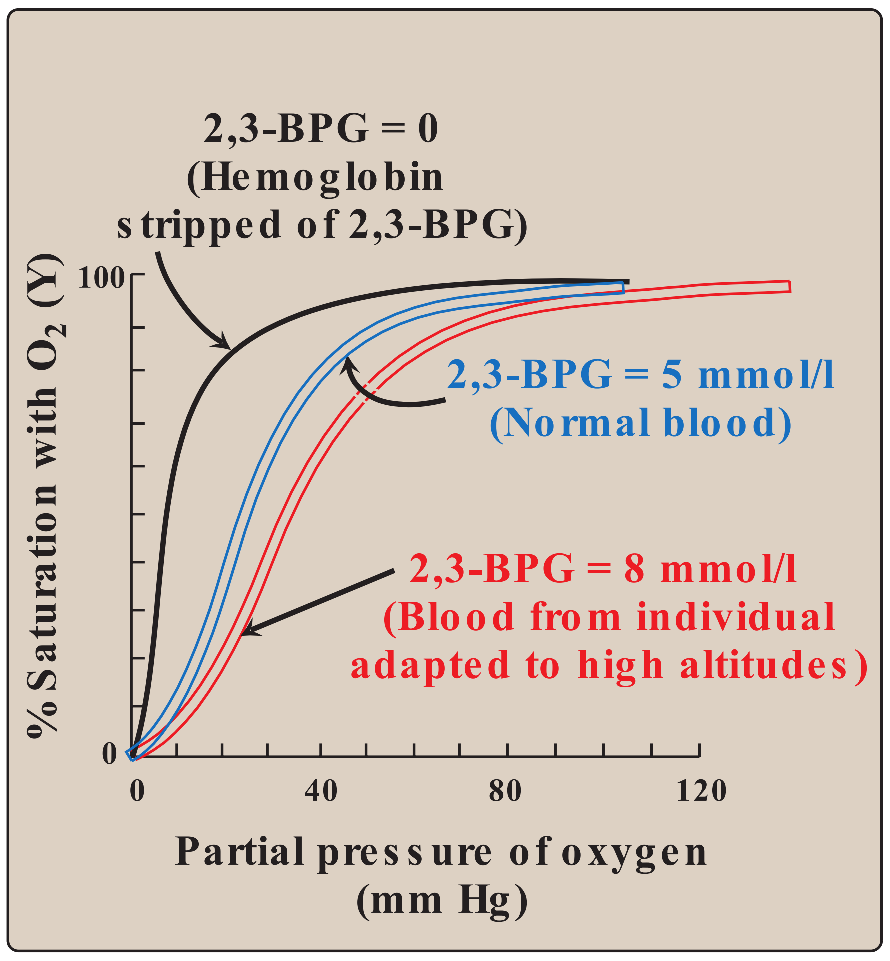


Figure 3.11
Allosteric effect of 2,3-bisphosphoglycerate (2,3-BPG) on the oxygen affinity of hemoglobin.

d. Response of 2,3-BPG levels to chronic hypoxia or anemia: The concentration of 2,3-BPG in the RBC increases in response to chronic hypoxia, such as that observed in chronic obstructive pulmonary disease (COPD) like emphysema, or at high altitudes, where circulating hemoglobin may have difficulty receiving sufficient oxygen. Intracellular levels of 2,3-BPG are also elevated in chronic anemia, in which fewer than normal RBCs are available to supply the body's oxygen needs. Elevated 2,3-BPG levels lower the oxygen affinity of hemoglobin, permitting greater unloading of oxygen in the capillaries of the tissues (see Figure 3.11).

e. Role of 2,3-BPG in transfused blood: 2,3-BPG is essential for the normal oxygen transport function of hemoglobin. However, storing blood in the currently available media results in a decrease in 2,3-BPG. Stored blood displays an abnormally high oxygen affinity and fails to unload its bound oxygen properly in the tissues. Hemoglobin deficient in 2,3-BPG thus acts as an oxygen "trap" rather than as an oxygen transport system. Transfused RBC are able to restore their depleted supplies of 2,3-BPG in 6–24 hours. However, severely ill patients may be compromised if transfused with large quantities of such 2,3-BPG–"stripped" blood. [Note: The maximum storage time for RBC has been doubled (21 to 42 days, with median time of 15 days) by changes in H^+, phosphate, and hexose sugar concentration and by the addition of adenine (see p. 291). Although the content of 2,3-BPG was not greatly improved in the long-term by these changes, adenosine triphosphate production was increased and improved RBC survival.]

4. Binding of CO_2: Most of the CO_2 produced in metabolism is hydrated and transported as bicarbonate ion (see p. 9). However, some CO_2 is carried as carbamate bound to the N-terminal amino groups of hemoglobin (forming carbaminohemoglobin as shown in Figure 3.7), which can be represented schematically as follows:

$$\text{Hb} - \text{NH}_2 + \text{CO}_2 \rightleftarrows \text{Hb} - \text{NH} - \text{COO}^- + \text{H}^+$$

The binding of CO_2 stabilizes the T or deoxy form of hemoglobin, resulting in a decrease in its affinity for oxygen (see p. 28) and a right shift in the oxygen-dissociation curve. In the lungs, CO_2 dissociates from the hemoglobin and is released in the breath.

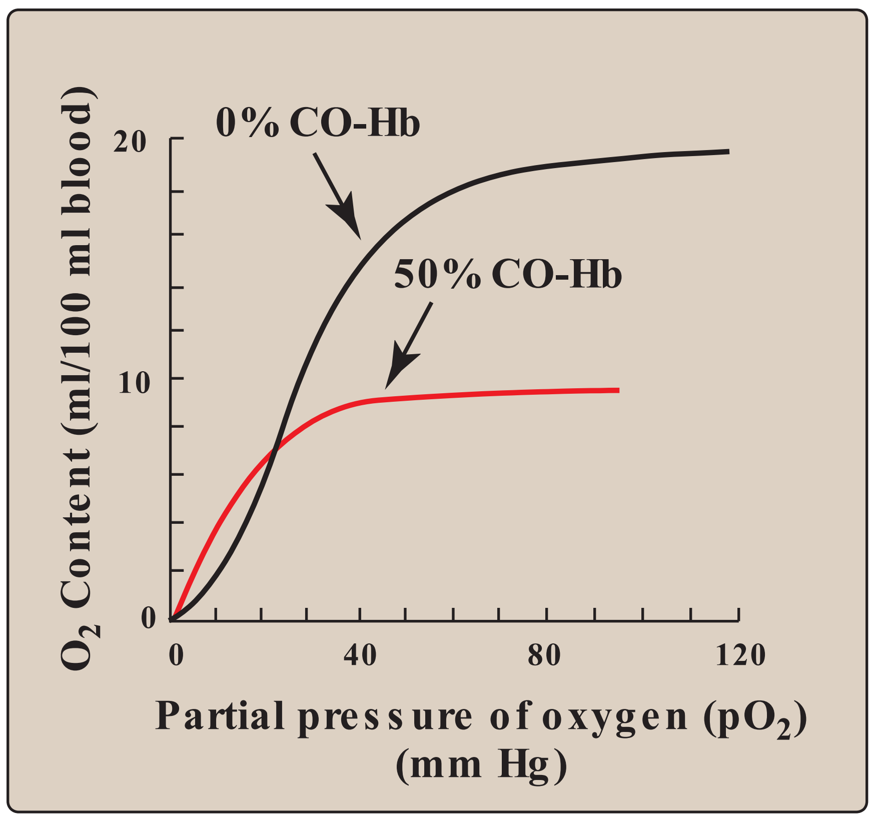


Figure 3.12
Effect of carbon monoxide (CO) on the oxygen affinity of hemoglobin. CO-Hb = carboxyhemoglobin (carbon monoxyhemoglobin).

5. Binding of CO: Carbon monoxide (CO) binds tightly (but reversibly) to the hemoglobin iron, forming carboxyhemoglobin. When CO binds to one or more of the four heme sites, hemoglobin shifts to the R conformation, causing the remaining heme sites to bind oxygen with high affinity. This shifts the oxygen-dissociation curve to the left and changes the normal sigmoidal shape toward a hyperbola. As a result, the affected hemoglobin is unable to release oxygen to the tissues (Figure 3.12). [Note: The affinity of hemoglobin for CO is 220 times greater than for oxygen. Consequently, even minute concentrations of CO in the environment can produce toxic concentrations of carboxyhemoglobin in the blood. For example, increased levels of CO are found in the blood of tobacco smokers. CO toxicity appears to result from a combination of tissue hypoxia and direct CO-mediated damage at the cellular level.] CO poisoning is treated with 100% oxygen at high pressure (hyperbaric oxygen therapy), which facilitates the dissociation of CO from the hemoglobin. [Note: CO inhibits Complex IV of the electron transport chain (see p. 76).] In addition to O_2, CO_2, and CO, nitric oxide gas (NO) also is carried by hemoglobin. NO is a potent vasodilator (see p. 151). It can be taken up (salvaged) or released from RBC, thus modulating NO availability and influencing vessel diameter.

Form	Chain composition	Fraction of total hemoglobin
HbA	$\alpha_2\beta_2$	90%
HbF	$\alpha_2\gamma_2$	<2%
HbA_2	$\alpha_2\delta_2$	2%–5%
HbA_{1c}	$\alpha_2\beta_2$-glucose	3%–9%

Figure 3.13
Normal adult human hemoglobins. [Note: The α-chains in these hemoglobins are identical.]
Hb = hemoglobin.

F. Minor hemoglobins

It is important to remember that human hemoglobin A (HbA) is just one member of a functionally and structurally related family of proteins, the hemoglobins (Figure 3.13). Each of these oxygen-carrying proteins is a tetramer, composed of two α-globin (or α-like) polypeptides and two β-globin (or β-like) polypeptides. Certain hemoglobins, such as HbF, are normally synthesized only during fetal development, whereas others, such as HbA_2, are synthesized in the adult, although at low levels compared with HbA. HbA can also become modified by the covalent addition of a hexose (see p. 34).

1. **Fetal hemoglobin:** HbF is a tetramer consisting of two α chains identical to those found in HbA, plus two γ chains ($\alpha_2\gamma_2$; see Figure 3.13). The γ chains are members of the β-globin gene family (see p. 35).

   a. **HbF synthesis during development:** In the first month after conception, embryonic hemoglobins such as Hb Gower 1, composed of two α-like zeta (ζ) chains and two β-like epsilon (ε) chains ($\zeta_2\varepsilon_2$), are synthesized by the embryonic yolk sac. In the fifth week of gestation, the site of globin synthesis shifts, first to the liver and then to the marrow, and the primary product is HbF. HbF is the major hemoglobin found in the fetus and newborn, accounting for about 60% of the total hemoglobin in the RBC during the last months of fetal life (Figure 3.14). HbA synthesis starts in the bone marrow at about the eighth month of pregnancy and gradually replaces HbF. (Figure 3.14 shows the relative production of each type of hemoglobin chain during fetal and postnatal life.) [Note: HbF represents less than 1% of the hemoglobin in most adults and is concentrated in RBC known as F cells.]

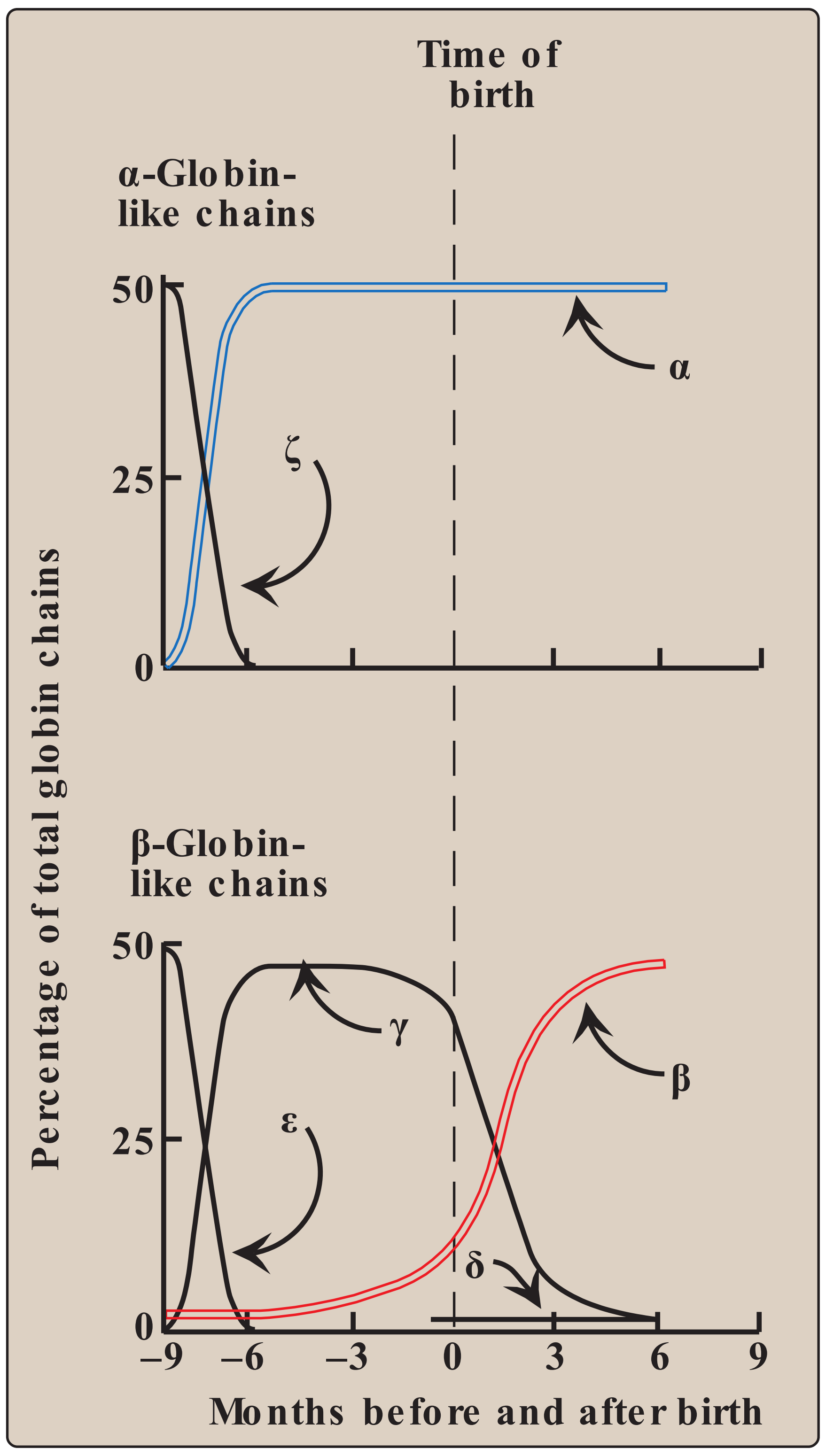


Figure 3.14
Developmental changes in hemoglobin.

   b. **Binding of 2,3-BPG to HbF:** Under physiologic conditions, HbF has a higher affinity for oxygen than does HbA as a result of HbF only weakly binding 2,3-BPG. [Note: The γ-globin chains of HbF lack some of the positively charged amino acids that are responsible for binding 2,3-BPG in the β-globin chains.] Because 2,3-BPG serves to reduce the affinity of hemoglobin for oxygen, the weaker interaction between 2,3-BPG and HbF results in a higher oxygen affinity for HbF relative to HbA. In contrast, if both HbA and HbF are stripped of their 2,3-BPG, they then have a similar affinity for oxygen. The higher oxygen affinity of HbF facilitates the transfer of oxygen from the maternal circulation across the placenta to the RBC of the fetus.

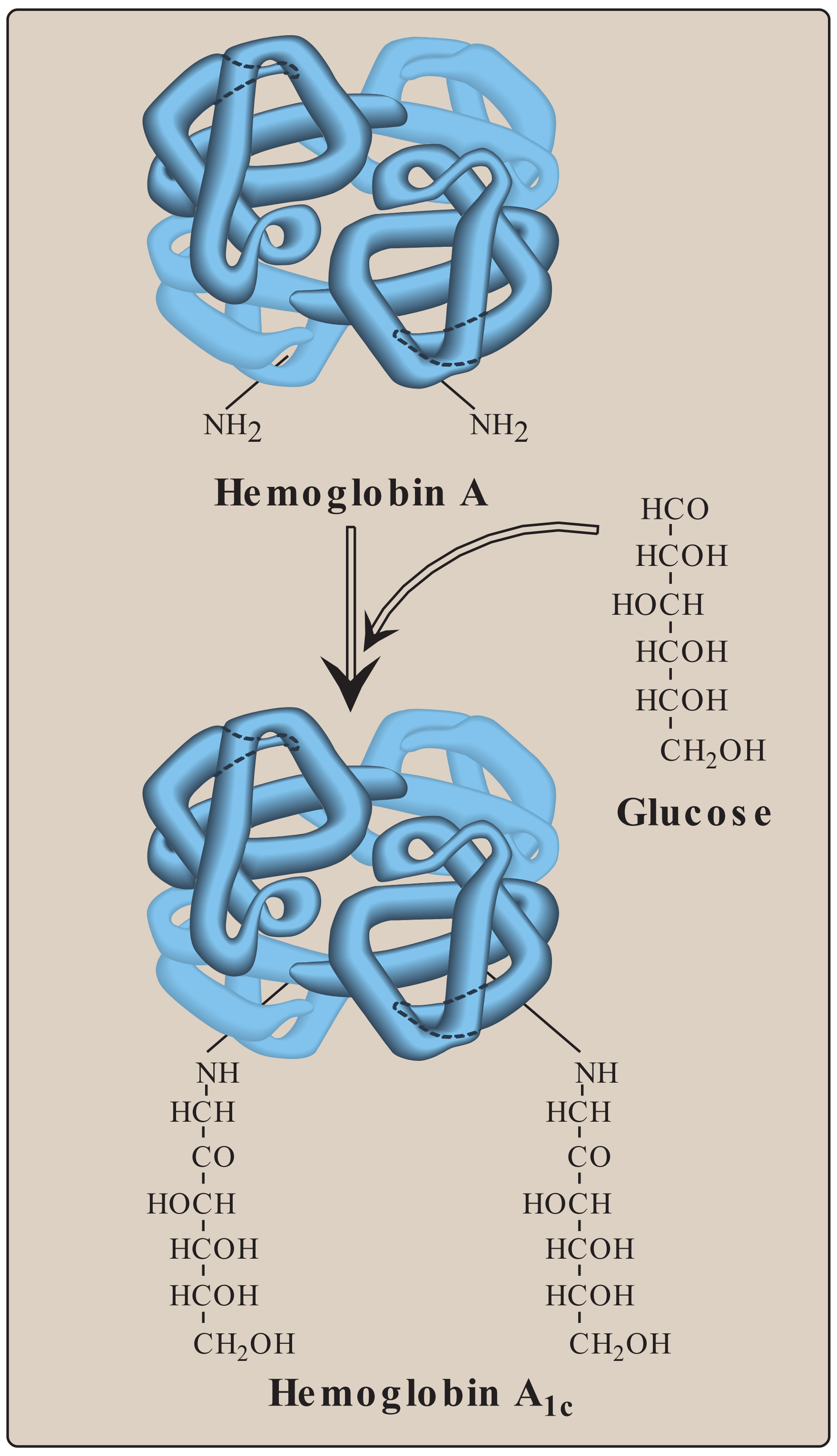

NH2
NH2
Hemoglobin A
HCO
HCOH
HOCH
HCOH
HCOH
CH2OH
Glucose
NH
HCH
CO
HOCH
HCOH
HCOH
CH2OH
NH
HCH
CO
HOCH
HCOH
HCOH
CH2OH
Hemoglobin A1c

Figure 3.15
Nonenzymic addition of glucose to hemoglobin. The nonenzymic addition of a sugar to a protein is referred to as glycation.

2. **Hemoglobin A_2:** HbA_2 is a minor component of normal adult hemoglobin, first appearing shortly before birth and, ultimately, constituting about 2% of the total hemoglobin. It is composed of two α-globin chains and two δ-globin chains ($\alpha_2\delta_2$; see Figure 3.13).

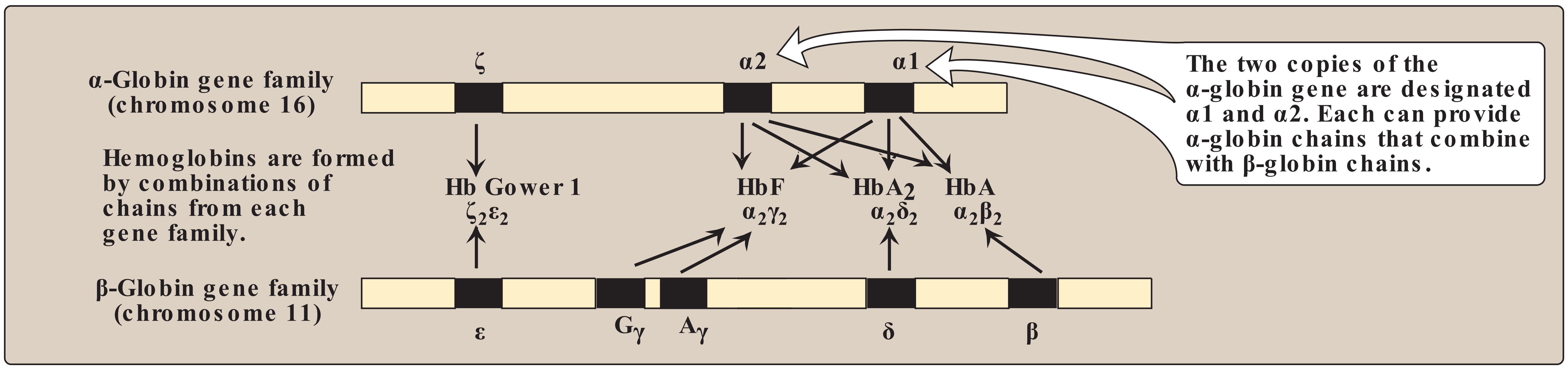
3. **Hemoglobin A_{1c}:** Under physiologic conditions, HbA is slowly and nonenzymically glycosylated (glycated), the extent of glycosylation being dependent on the plasma concentration of a particular hexose. The most abundant form of glycosylated hemoglobin is HbA_{1c}. It has glucose residues attached predominantly to the NH_2 groups of the N-terminal valines of the β-globin chains (Figure 3.15). Increased amounts of HbA_{1c} are found in RBC of patients with diabetes mellitus, because their HbA has contact with higher glucose concentrations during the 120-day lifetime of these cells. (See p. 340 for a discussion of the use of HbA_{1c} levels in assessing average blood glucose levels in patients with diabetes.)

III. ORGANIZATION OF THE GLOBIN GENES

To understand diseases resulting from genetic alterations in the structure or synthesis of hemoglobins, it is necessary to grasp how the hemoglobin genes, which direct the synthesis of the different globin chains, are structurally organized into gene families and also how they are expressed.

A. α-Gene family

The genes coding for the α-globin and β-globin subunits of the hemoglobin chains occur in two separate gene clusters (or families) located on two different chromosomes (Figure 3.16). The α-gene cluster on chromosome 16 contains two genes for the α-globin chains. It also contains the ζ gene that is expressed early in development as an α-globin-like component of embryonic hemoblobin. [Note: Globin gene famillies also contain globin-like genes that are not expressed, that is, their genetic information is not used to produce globin chains. These are called pseudogenes.]


α-Globin gene family (chromosome 16)
ζ
α2
α1
Hb Gower 1
ζ2ε2
HbF
α2γ2
HbA2
α2δ2
HbA
α2β2
β-Globin gene family (chromosome 11)
ε
Gγ
Aγ
δ
β

Hemoglobins are formed by combinations of chains from each gene family.

The two copies of the α-globin gene are designated α1 and α2. Each can provide α-globin chains that combine with β-globin chains.

Figure 3.16
Organization of the globin gene families. Hb = hemoglobin.

B. β-Gene family

A single gene for the β-globin chain is located on chromosome 11 (see Figure 3.16). There are an additional four β-globin-like genes: the ε gene (which, like the ζ gene, is expressed early in embryonic development), two γ genes (G_γ and A_γ that are expressed in HbF), and the δ gene that codes for the globin chain found in the minor adult hemoglobin HbA_2.

C. Steps in globin chain synthesis

Expression of a globin gene begins in the nucleus of RBC precursors, where the DNA sequence encoding the gene is transcribed. The RNA produced by transcription is actually a precursor of the messenger RNA (mRNA) that is used as a template for the synthesis of a globin chain. Before it can serve this function, two noncoding stretches of RNA (introns) must be removed from the mRNA precursor sequence and the remaining three fragments (exons) joined in a linear manner. The resulting mature mRNA enters the cytosol, where its genetic information is translated, producing a globin chain. (A summary of this process is shown in Figure 3.17. A more detailed description of gene expresion is presented in Unit VI, p. 395.)

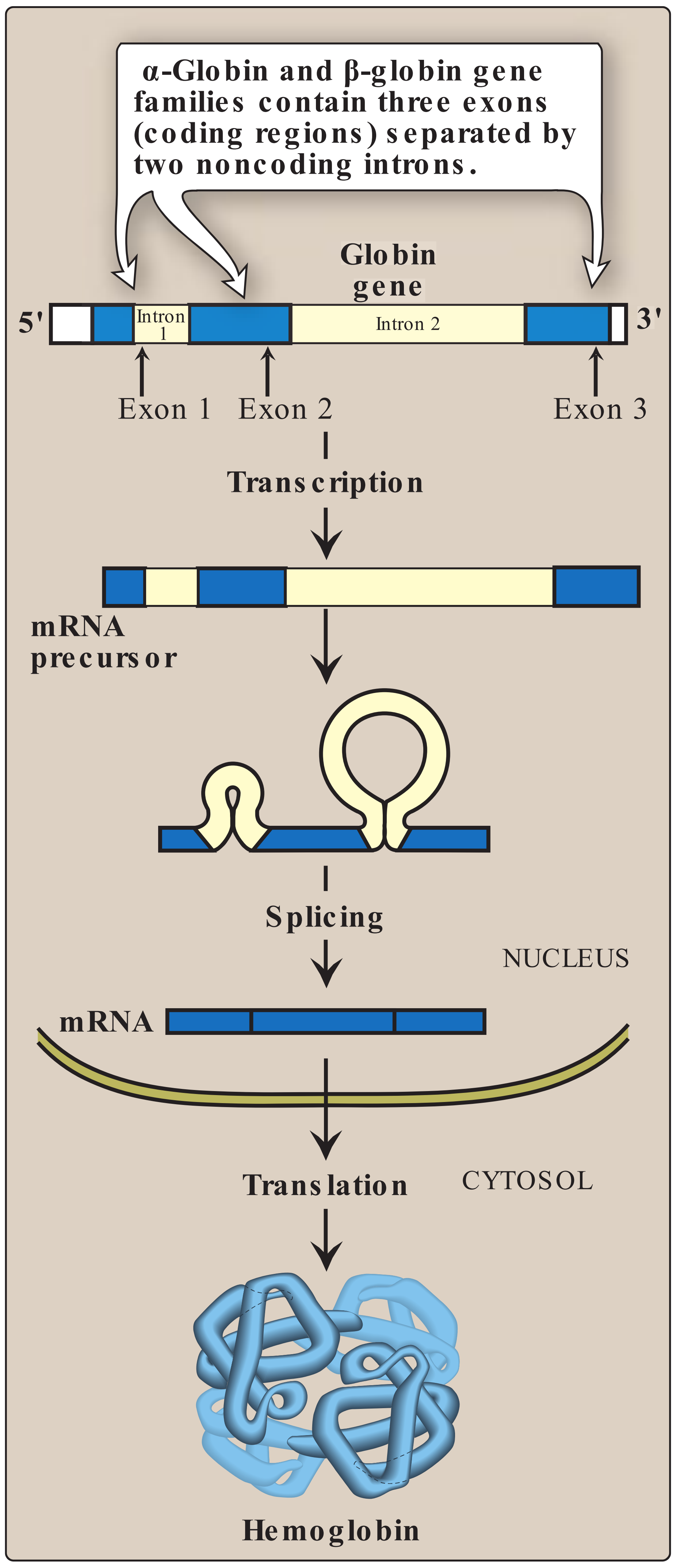


Figure 3.17
Synthesis of globin chains. mRNA = messenger RNA.

IV. HEMOGLOBINOPATHIES

Hemoglobinopathies are defined as a group of genetic disorders caused by production of a structurally abnormal hemoglobin molecule; synthesis of insufficient quantities of normal hemoglobin; or, rarely, both. Sickle cell anemia (HbS), hemoglobin C disease (HbC), hemoglobin SC disease (HbS + HbC = HbSC), and the thalassemias are representative hemoglobinopathies that can have severe clinical consequences. The first three conditions result from production of hemoglobin with an altered amino acid sequence (qualitative hemoglobinopathy), whereas the thalassemias are caused by decreased production of normal hemoglobin (quantitative hemoglobinopathy).

A. Sickle cell anemia (hemoglobin S disease)

Sickle cell anemia, the most common of the RBC sickling diseases, is a genetic disorder of the blood caused by a single nucleotide substitution (a point mutation, see p. 433) in the gene for β-globin. It is the most common inherited blood disorder in the United States, affecting 50,000 Americans. It occurs primarily in the African American population, affecting one of 500 newborn African American infants in the United States. Sickle cell anemia is an autosomal recessive disorder. It occurs in individuals who have inherited two mutant genes (one from each parent) that code for synthesis of the β chains of the globin molecules. [Note: The mutant β-globin chain is designated β^S, and the resulting hemoglobin, $\alpha_2\beta^S{}_2$, is referred to as HbS.] An infant does not begin showing symptoms of the disease until sufficient HbF has been replaced by HbS so that sickling can occur (see below). Sickle cell anemia is characterized by lifelong episodes of pain ("crises"); chronic hemolytic anemia with associated hyperbilirubinemia (see p. 284); and increased susceptibility to infections, usually beginning in infancy. [Note: The lifetime of a RBC in sickle cell anemia is less than 20 days, compared with 120 days

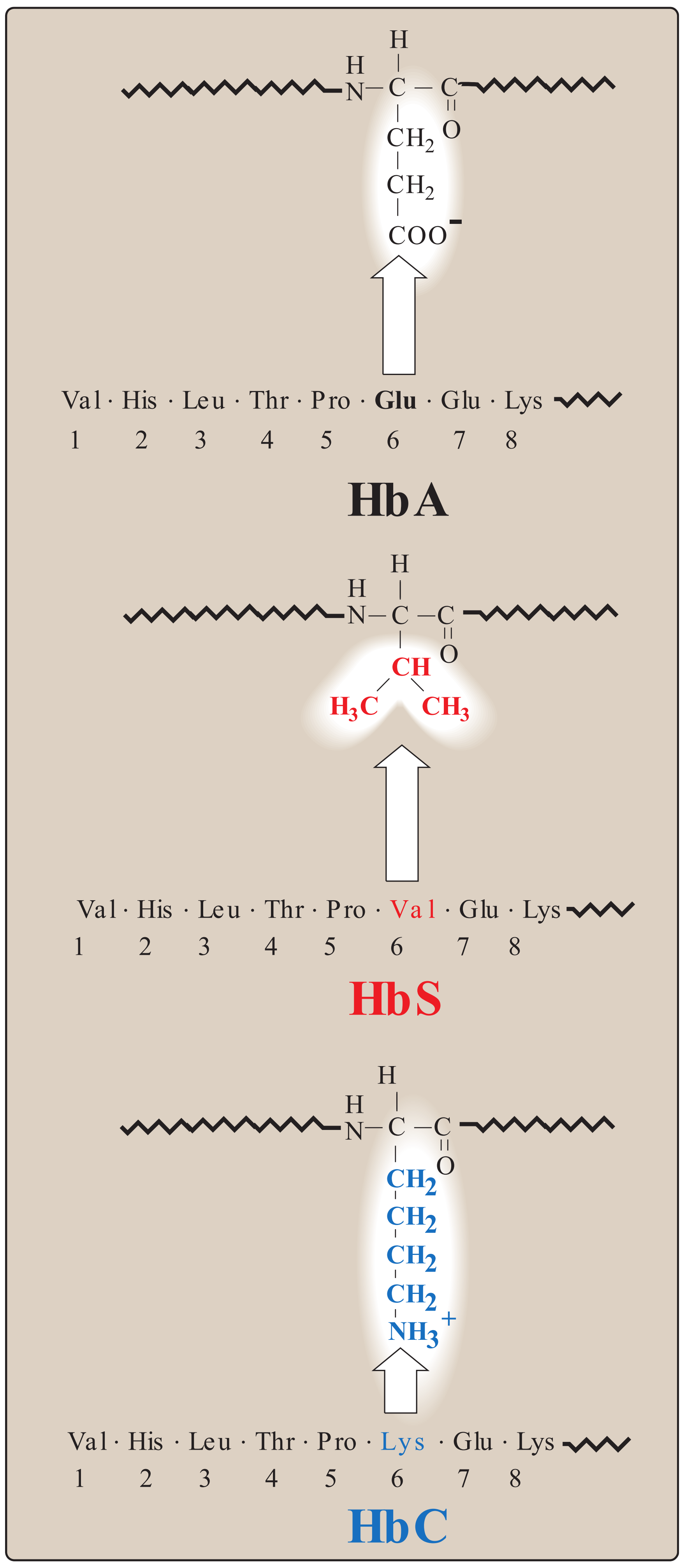

H
H
N–C–C
CH₂ O
CH₂
COO⁻
Val · His · Leu · Thr · Pro · Glu · Glu · Lys
1 2 3 4 5 6 7 8
HbA
CH
H₃C CH₃
Val · His · Leu · Thr · Pro · Val · Glu · Lys
1 2 3 4 5 6 7 8
HbS
CH₂
CH₂
CH₂
CH₂
NH₃⁺
Val · His · Leu · Thr · Pro · Lys · Glu · Lys
1 2 3 4 5 6 7 8
HbC

Figure 3.18
Amino acid substitutions in hemoglobin S (HbS) and hemoglobin C (HbC).

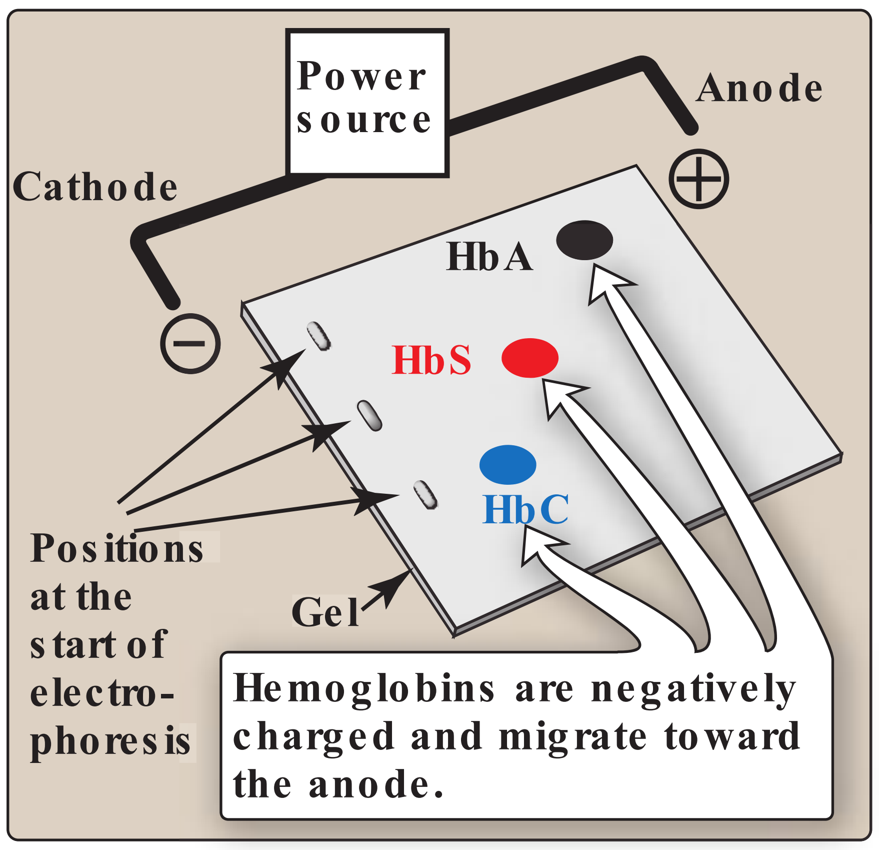

Power source
Anode
Cathode
HbA
HbS
HbC
Positions at the start of electro-phoresis
Gel
Hemoglobins are negatively charged and migrate toward the anode.

Figure 3.19
Diagram of hemoglobins (HbA), (HbS), and (HbC) after electrophoresis.

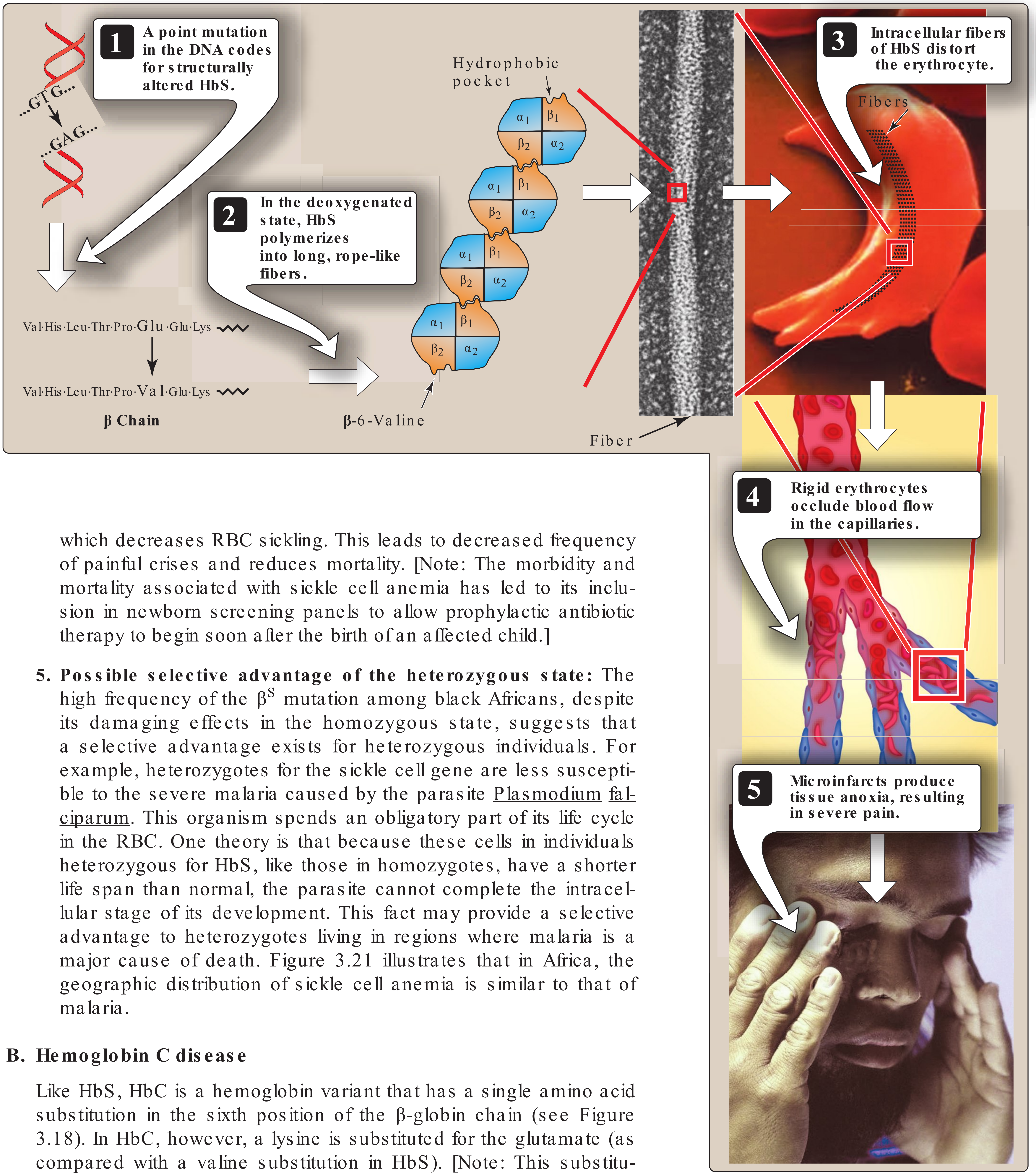
for normal RBC, hence, the anemia.] Other symptoms include acute chest syndrome, stroke, splenic and renal dysfunction, and bone changes due to marrow hyperplasia. Heterozygotes, representing 1 in 12 African Americans, have one normal and one sickle cell gene. The blood cells of such heterozygotes contain both HbS and HbA. These individuals have sickle cell trait. They usually do not show clinical symptoms (but may under conditions of extreme physical exertion with dehydration) and can have a normal life span.

1. **Amino acid substitution in HbS β chains:** A molecule of HbS contains two normal α-globin chains and two mutant β-globin chains (β^S), in which glutamate at position six has been replaced with valine (Figure 3.18). Therefore, during electrophoresis at alkaline pH, HbS migrates more slowly toward the anode (positive electrode) than does HbA (Figure 3.19). This altered mobility of HbS is a result of the absence of the negatively charged glutamate residues in the two β chains, thereby rendering HbS less negative than HbA. [Note: Electrophoresis of hemoglobin obtained from lysed RBC is routinely used in the diagnosis of sickle cell trait and sickle cell disease. DNA analysis also is used (see p. 472).]

2. **Sickling and tissue anoxia:** The replacement of the charged glutamate with the nonpolar valine forms a protrusion on the β chain that fits into a complementary site on the β chain of another hemoglobin molecule in the cell (Figure 3.20). At low oxygen tension, deoxyhemoglobin S polymerizes inside the RBC, forming a network of insoluble fibrous polymers that stiffen and distort the cell, producing rigid, misshapen RBC. Such sickled cells frequently block the flow of blood in the narrow capillaries. This interruption in the supply of oxygen leads to localized anoxia (oxygen deprivation) in the tissue, causing pain and eventually death (infarction) of cells in the vicinity of the blockage. The anoxia also leads to an increase in deoxygenated HbS. [Note: The mean diameter of RBC is 7.5 µm, whereas that of the microvasculature is 3–4 µm. Compared to normal RBC, sickled cells have a decreased ability to deform and an increased tendency to adhere to vessel walls and so have difficulty moving through small vessels, thereby causing microvascular occlusion.]

3. **Variables that increase sickling:** The extent of sickling and, therefore, the severity of disease is enhanced by any variable that increases the proportion of HbS in the deoxy state (that is, reduces the affinity of HbS for O_2). These variables include decreased pO_2, increased pCO_2, decreased pH, dehydration, and an increased concentration of 2,3-BPG in RBC.

4. **Treatment:** Therapy involves adequate hydration, analgesics, aggressive antibiotic therapy if infection is present, and transfusions in patients at high risk for fatal occlusion of blood vessels. Intermittent transfusions with packed RBC reduce the risk of stroke, but the benefits must be weighed against the complications of transfusion, which include iron overload (hemosiderosis), bloodborne infections, and immunologic complications. Hydroxyurea (hydroxycarbamide), an antitumor drug, is therapeutically useful because it increases circulating levels of HbF,



1 A point mutation in the DNA codes for structurally altered HbS.

2 In the deoxygenated state, HbS polymerizes into long, rope-like fibers.

3 Intracellular fibers of HbS distort the erythrocyte.

4 Rigid erythrocytes occlude blood flow in the capillaries.

5 Microinfarcts produce tissue anoxia, resulting in severe pain.

Figure 3.20
Molecular and cellular events leading to sickle cell crisis. HbS = hemoglobin S.

which decreases RBC sickling. This leads to decreased frequency of painful crises and reduces mortality. [Note: The morbidity and mortality associated with sickle cell anemia has led to its inclusion in newborn screening panels to allow prophylactic antibiotic therapy to begin soon after the birth of an affected child.]

5. **Possible selective advantage of the heterozygous state:** The high frequency of the β^S mutation among black Africans, despite its damaging effects in the homozygous state, suggests that a selective advantage exists for heterozygous individuals. For example, heterozygotes for the sickle cell gene are less susceptible to the severe malaria caused by the parasite Plasmodium falciparum. This organism spends an obligatory part of its life cycle in the RBC. One theory is that because these cells in individuals heterozygous for HbS, like those in homozygotes, have a shorter life span than normal, the parasite cannot complete the intracellular stage of its development. This fact may provide a selective advantage to heterozygotes living in regions where malaria is a major cause of death. Figure 3.21 illustrates that in Africa, the geographic distribution of sickle cell anemia is similar to that of malaria.

B. Hemoglobin C disease

Like HbS, HbC is a hemoglobin variant that has a single amino acid substitution in the sixth position of the β-globin chain (see Figure 3.18). In HbC, however, a lysine is substituted for the glutamate (as compared with a valine substitution in HbS). [Note: This substitution causes HbC to move more slowly toward the anode than HbA or HbS does (see Figure 3.19).] Rare patients homozygous for HbC generally have a relatively mild, chronic hemolytic anemia. These patients do not suffer from infarctive crises, and no specific therapy is required.

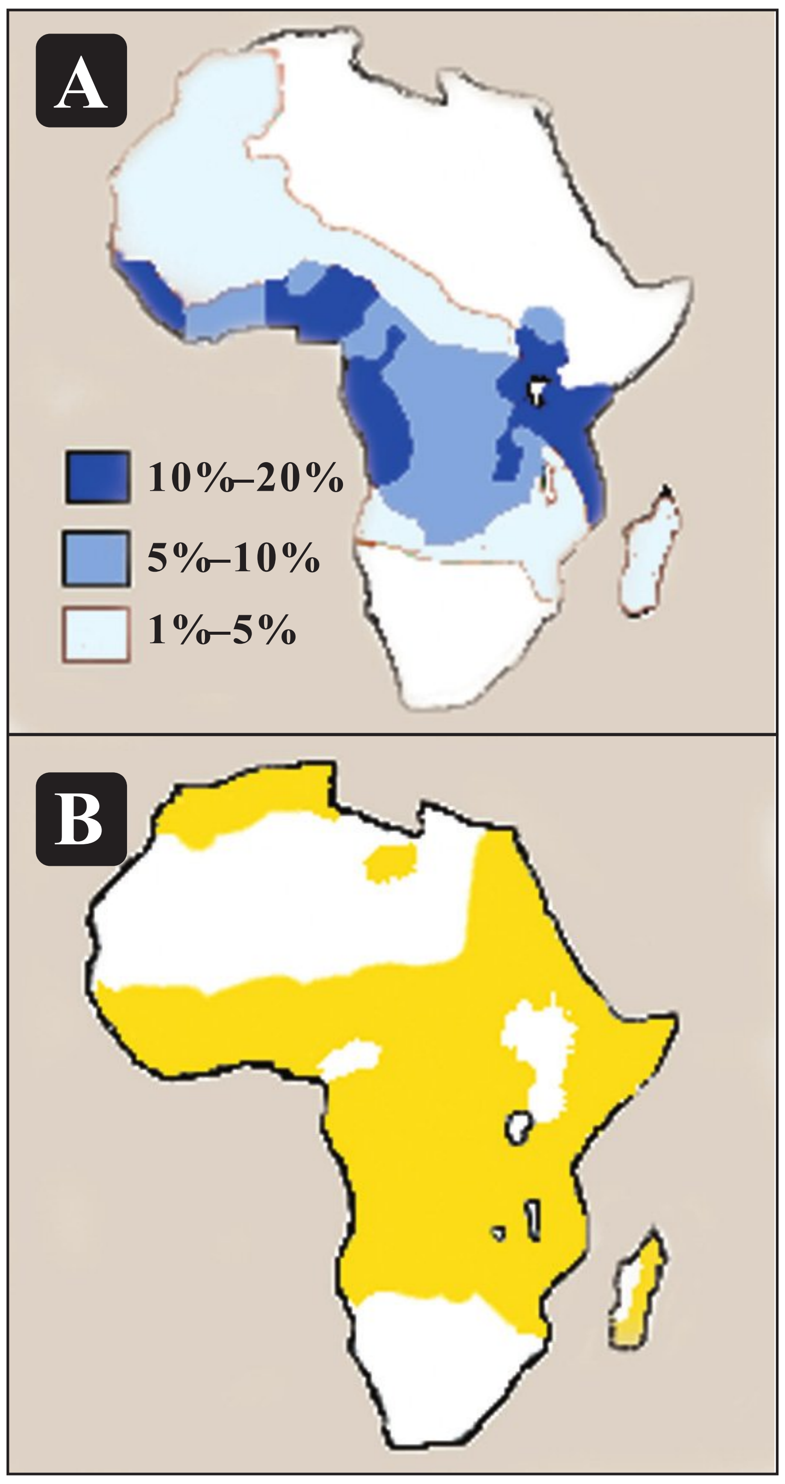


Figure 3.21
A. Distribution of sickle cell in Africa expressed as a percentage of the population with disease.
B. Distribution of malaria in Africa.

C. Hemoglobin SC disease

HbSC disease is another of the RBC sickling diseases. In this disease, some β-globin chains have the sickle cell mutation, whereas other β-globin chains carry the mutation found in HbC disease. [Note: Patients with HbSC disease are doubly heterozygous. They are called compound heterozygotes because both of their β-globin genes are abnormal, although different from each other.] Hemoglobin levels tend to be higher in HbSC disease than in sickle cell anemia and may even be at the low end of the normal range. The clinical course of adults with HbSC anemia differs from that of sickle cell anemia in that symptoms such as painful crises are less frequent and less severe. However, there is significant clinical variability.

D. Methemoglobinemias

Oxidation of the heme iron in hemoglobin to the ferric (Fe^{3+}) state forms methemoglobin, which cannot bind O_2. This oxidation may be caused by the action of certain drugs, such as nitrates, or endogenous products such as reactive oxygen species (see p. 148). The oxidation may also result from inherited defects, for example, certain mutations in the α- or β-globin chain promote the formation of methemoglobin (HbM). Additionally, a deficiency of NADH-cytochrome b_5 reductase (also called NADH-methemoglobin reductase), the enzyme responsible for the conversion of methemoglobin (Fe^{3+}) to hemoglobin (Fe^{2+}), leads to the accumulation of HbM. [Note: The RBC of newborns have approximately half the capacity of those of adults to reduce HbM. They are, therefore, particularly susceptible to the effects of HbM-producing compounds.] The methemoglobinemias are characterized by "chocolate cyanosis" (a brownish blue coloration of the skin and mucous membranes and brown-colored blood) as a result of the dark-colored HbM. Symptoms are related to the degree of tissue hypoxia and include anxiety, headache, and dyspnea. In rare cases, coma and death can occur. Treatment is with methylene blue, which is oxidized as Fe^{+3} is reduced.

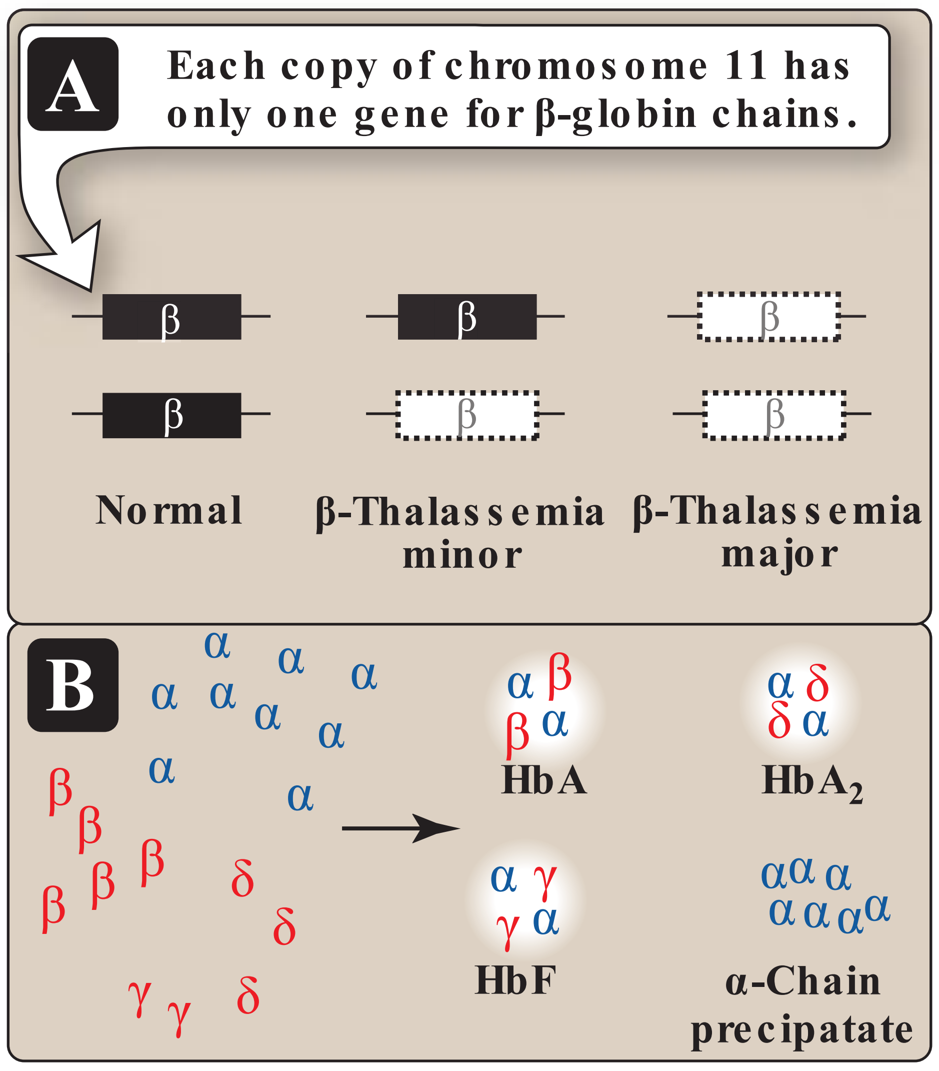


Figure 3.22
A. β-Globin gene mutations in the β-thalassemias. B. Hemoglobin (Hb) tetramers formed in β-thalassemias.

E. Thalassemias

The thalassemias are hereditary hemolytic diseases in which an imbalance occurs in the synthesis of globin chains. As a group, they are the most common single gene disorders in humans. Normally, synthesis of the α- and β-globin chains is coordinated, so that each α-globin chain has a β-globin chain partner. This leads to the formation of $\alpha_2\beta_2$ (HbA). In the thalassemias, the synthesis of either the α- or the β-globin chain is defective. A thalassemia can be caused by a variety of mutations, including entire gene deletions, or substitutions or deletions of one to many nucleotides in the DNA. [Note: Each thalassemia can be classified as either a disorder in which no globin chains are produced (α^o- or β^o-thalassemia), or one in which some chains are synthesized but at a reduced level (α^+- or β^+-thalassemia).]

1. **β-Thalassemias:** In these disorders, synthesis of β-globin chains is decreased or absent, typically as a result of point mutations that affect the production of functional mRNA. However, α-globin chain synthesis is normal. Excess α-globin chains cannot form stable tetramers and so precipitate, causing the premature death of cells initially destined to become mature RBC. Increase in

$\alpha_2\delta_2$ (HbA$_2$) and $\alpha_2\gamma_2$ (HbF) also occurs. There are only two copies of the β-globin gene in each cell (one on each chromosome 11). Therefore, individuals with β-globin gene defects have either β-thalassemia trait (β-thalassemia minor) if they have only one defective β-globin gene or β-thalassemia major (Cooley anemia) if both genes are defective (Figure 3.22). Because the β-globin gene is not expressed until late in fetal gestation, the physical manifestations of β-thalassemias appear only several months after birth. Those individuals with β-thalassemia minor make some β chains, and usually do not require specific treatment. However, those infants born with β-thalassemia major are seemingly healthy at birth but become severely anemic, usually during the first or second year of life due to ineffective erythropoiesis. Skeletal changes as a result of extramedullary hematopoiesis also are seen.These patients require regular transfusions of blood. [Note: Although this treatment is lifesaving, the cumulative effect of the transfusions is iron overload (a syndrome known as hemosiderosis). Use of iron chelation therapy has improved morbidity and mortality.] The only curative option available is hematopoietic stem cell transplantation.

2. **α-Thalassemias:** In these disorders, synthesis of α-globin chains is decreased or absent, typically as a result of deletional mutations. Because each individual's genome contains four copies of the α-globin gene (two on each chromosome 16), there are several levels of α-globin chain deficiencies (Figure 3.23). If one of the four genes is defective, the individual is termed a silent carrier of α-thalassemia, because no physical manifestations of the disease occur. If two α-globin genes are defective, the individual is designated as having α-thalassemia trait. If three α-globin genes are defective, the individual has hemoglobin H (β_4) disease, a hemolytic anemia of variable severity. If all four α-globin genes are defective, hemoglobin Bart (γ_4) disease with hydrops fetalis and fetal death results, because α-globin chains are required for the synthesis of HbF.

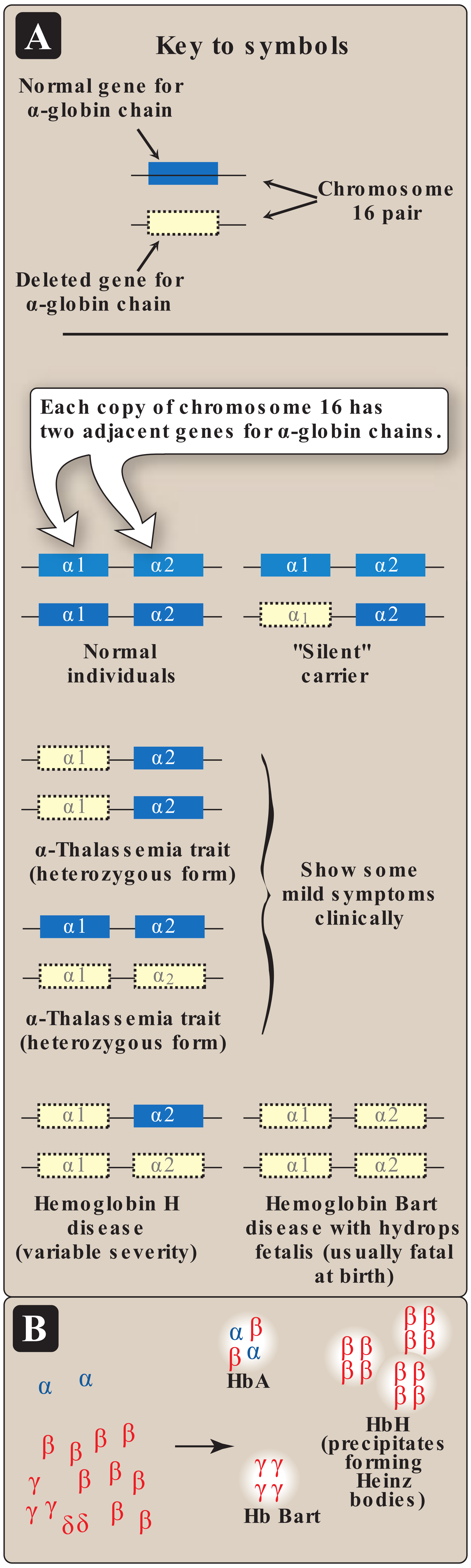

A
Key to symbols
Normal gene for α-globin chain
Chromosome 16 pair
Deleted gene for α-globin chain
Each copy of chromosome 16 has two adjacent genes for α-globin chains.
α1
α2
Normal individuals
"Silent" carrier
α-Thalassemia trait (heterozygous form)
α-Thalassemia trait (heterozygous form)
Show some mild symptoms clinically
Hemoglobin H disease (variable severity)
Hemoglobin Bart disease with hydrops fetalis (usually fatal at birth)
B
α
β
γ
δ
HbA
HbH (precipitates forming Heinz bodies)
Hb Bart

Figure 3.23
A. α-Globin gene deletions in the α-thalassemias. B. Hemoglobin (Hb) tetramers formed in α-thalassemias.

V. CHAPTER SUMMARY

Hemoglobin A (HbA), the major hemoglobin (Hb) in adults, is composed of four polypeptide chains (two α chains and two β chains, $\boldsymbol{\alpha_2\beta_2}$) held together by noncovalent interactions (Figure 3.24). The subunits occupy different relative positions in deoxyhemoglobin compared with oxyhemoglobin. The **deoxy form** of Hb is called the **"T,"** or **taut (tense) conformation**. It has a constrained structure that limits the movement of the polypeptide chains. The T form is the **low-oxygen-affinity form** of Hb. The binding of O_2 to Hb causes rupture of some of the ionic and hydrogen bonds, and movement of the dimers. This leads to a structure called the **"R,"** or **relaxed conformation**. The R form is the **high-oxygen-affinity form** of Hb. The **oxygen-dissociation curve** for Hb is **sigmoidal** in shape (in contrast to that of **myoglobin**, which is **hyperbolic**), indicating that the subunits cooperate in binding O_2. **Cooperative binding** of O_2 by the four subunits of Hb means that the binding of an O_2 molecule at one heme group increases the oxygen affinity of the remaining heme groups in the same Hb molecule. Hb's **ability to bind O_2 reversibly** is affected by the **partial pressure of O_2 (pO_2)** (through heme-heme interactions), the **pH** of the environment, the partial pressure of CO_2 (**pCO_2**), and the availability of **2,3-bisphosphoglycerate** (**2,3-BPG**). For example, the release of O_2 from Hb is enhanced when the pH is lowered or the pCO_2 is increased (the **Bohr effect**), such as in **exercising muscle**, and the oxygen-dissociation curve of Hb is shifted to the right. To cope long-term with the effects of **chronic hypoxia** or **anemia**, the concentration of **2,3-BPG** in **red blood cells** increases. **2,3-BPG** binds to the Hb and decreases its oxygen affinity. It therefore also shifts the oxygen-dissociation curve to the right. **Carbon monoxide** (**CO**) binds tightly (but reversibly) to the Hb iron, forming **carboxyhemoglobin**. **Hemoglobinopathies** are disorders caused either by production of a **structurally abnormal Hb** molecule; synthesis of **insufficient quantities** of normal Hb subunits, or, rarely, both (Figure 3.25). The sickling diseases **sickle cell anemia** (hemoglobin S disease) and **hemoglobin SC disease** as well as **hemoglobin C disease** and the **thalassemias** are representative hemoglobinopathies that can have severe clinical consequences.

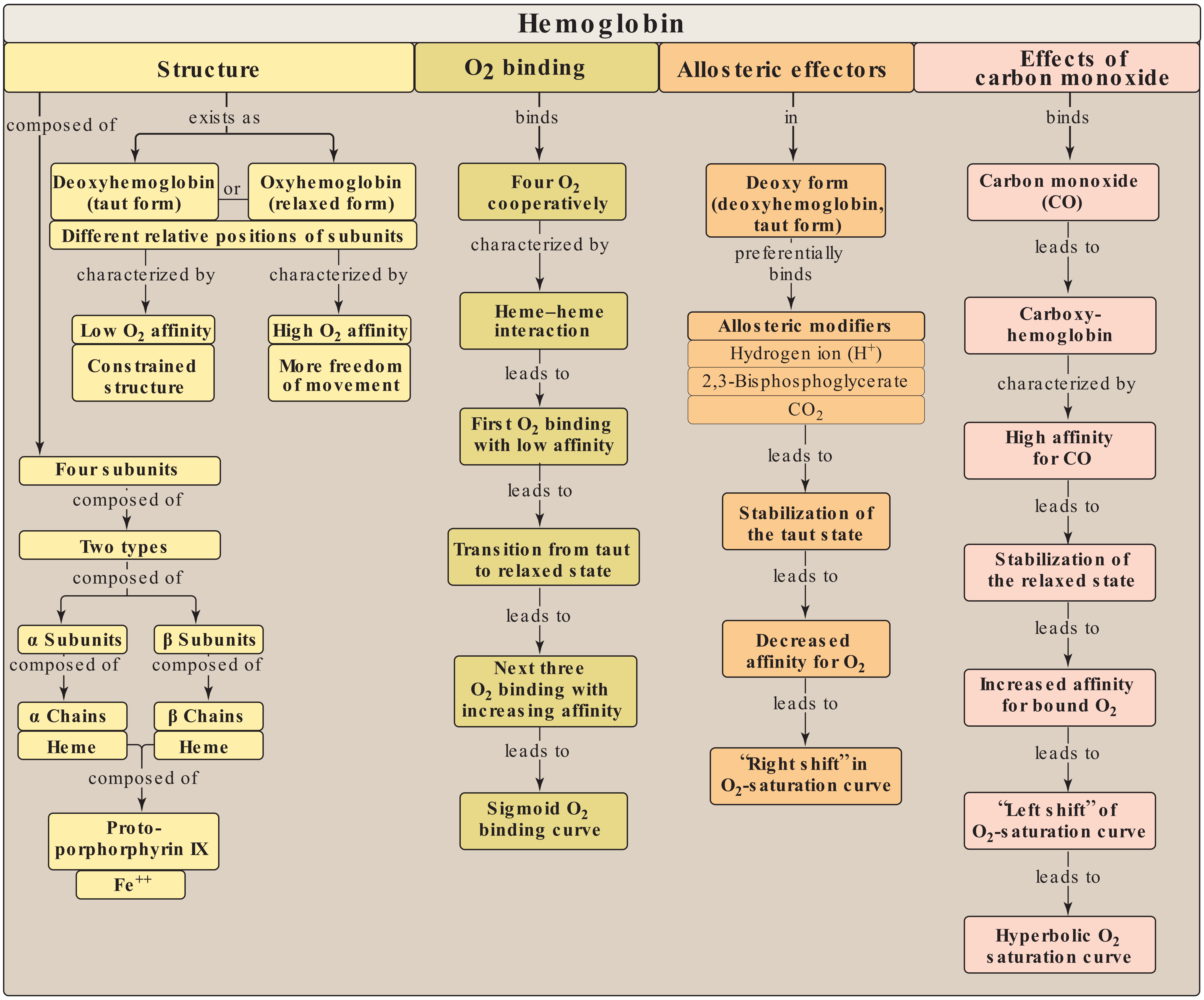

Hemoglobin
Structure
O_2 binding
Allosteric effectors
Effects of carbon monoxide
composed of
exists as
Deoxyhemoglobin (taut form)
or
Oxyhemoglobin (relaxed form)
Different relative positions of subunits
characterized by
characterized by
Low O_2 affinity
Constrained structure
High O_2 affinity
More freedom of movement
Four subunits
composed of
Two types
composed of
α Subunits
β Subunits
composed of
composed of
α Chains
β Chains
Heme
Heme
composed of
Proto-porphorphyrin IX
Fe^{++}
binds
Four O_2 cooperatively
characterized by
Heme–heme interaction
leads to
First O_2 binding with low affinity
leads to
Transition from taut to relaxed state
leads to
Next three O_2 binding with increasing affinity
leads to
Sigmoid O_2 binding curve
in
Deoxy form (deoxyhemoglobin, taut form)
preferentially binds
Allosteric modifiers
Hydrogen ion (H^+)
2,3-Bisphosphoglycerate
CO_2
leads to
Stabilization of the taut state
leads to
Decreased affinity for O_2
leads to
"Right shift" in O_2-saturation curve
binds
Carbon monoxide (CO)
leads to
Carboxy-hemoglobin
characterized by
High affinity for CO
leads to
Stabilization of the relaxed state
leads to
Increased affinity for bound O_2
leads to
"Left shift" of O_2-saturation curve
leads to
Hyperbolic O_2 saturation curve

Figure 3.24

Key concept map for hemoglobin structure and function.

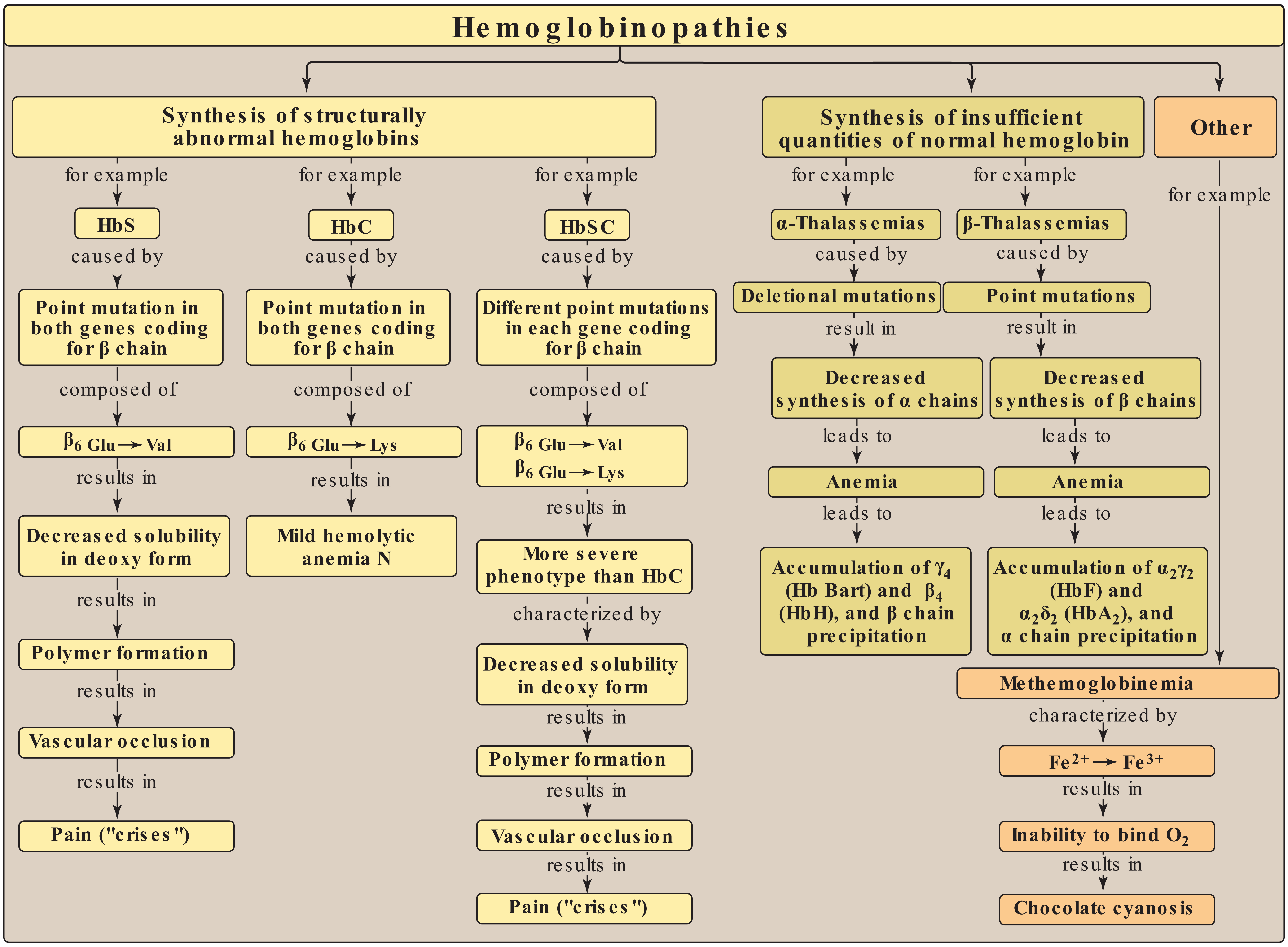

Hemoglobinopathies
Synthesis of structurally abnormal hemoglobins
for example
HbS
caused by
Point mutation in both genes coding for β chain
composed of
β_6 Glu → Val
results in
Decreased solubility in deoxy form
results in
Polymer formation
results in
Vascular occlusion
results in
Pain ("crises")
for example
HbC
caused by
Point mutation in both genes coding for β chain
composed of
β_6 Glu → Lys
results in
Mild hemolytic anemia N
for example
HbSC
caused by
Different point mutations in each gene coding for β chain
composed of
β_6 Glu → Val
β_6 Glu → Lys
results in
More severe phenotype than HbC
characterized by
Decreased solubility in deoxy form
results in
Polymer formation
results in
Vascular occlusion
results in
Pain ("crises")
Synthesis of insufficient quantities of normal hemoglobin
for example
α-Thalassemias
caused by
Deletional mutations
result in
Decreased synthesis of α chains
leads to
Anemia
leads to
Accumulation of γ_4 (Hb Bart) and β_4 (HbH), and β chain precipitation
for example
β-Thalassemias
caused by
Point mutations
result in
Decreased synthesis of β chains
leads to
Anemia
leads to
Accumulation of $\alpha_2\gamma_2$ (HbF) and $\alpha_2\delta_2$ (HbA_2), and α chain precipitation
Other
for example
Methemoglobinemia
characterized by
$Fe^{2+} \rightarrow Fe^{3+}$
results in
Inability to bind O_2
results in
Chocolate cyanosis

Figure 3.25
Key concept map for hemoglobinopathies. Hb = hemoglobin.

Fibrous Proteins

4

I. OVERVIEW

Collagen and elastin are examples of common, well-characterized fibrous proteins of the extracellular matrix that serve structural functions in the body. For example, collagen and elastin are found as components of skin, connective tissue, blood vessel walls, and the sclera and cornea of the eye. Each fibrous protein exhibits special mechanical properties, resulting from its unique structure, which are obtained by combining specific amino acids into regular, secondary structural elements. This is in contrast to globular proteins, whose shapes are the result of complex interactions between secondary, tertiary, and, sometimes, quaternary structural elements.

II. COLLAGEN

Collagen is the most abundant protein in the human body. A typical collagen molecule is a long, rigid structure in which three polypeptides (referred to as α chains) are wound around one another in a rope-like triple helix (Figure 4.1). Although these molecules are found throughout the body, their types and organization are dictated by the structural role collagen plays in a particular organ. In some tissues, collagen may be dispersed as a gel that gives support to the structure, as in the extracellular matrix or the vitreous humor of the eye. In other tissues, collagen may be bundled in tight, parallel fibers that provide great strength, as in tendons. In the cornea of the eye, collagen is stacked so as to transmit light with a minimum of scattering. Collagen of bone occurs as fibers arranged at an angle to each other so as to resist mechanical shear from any direction.

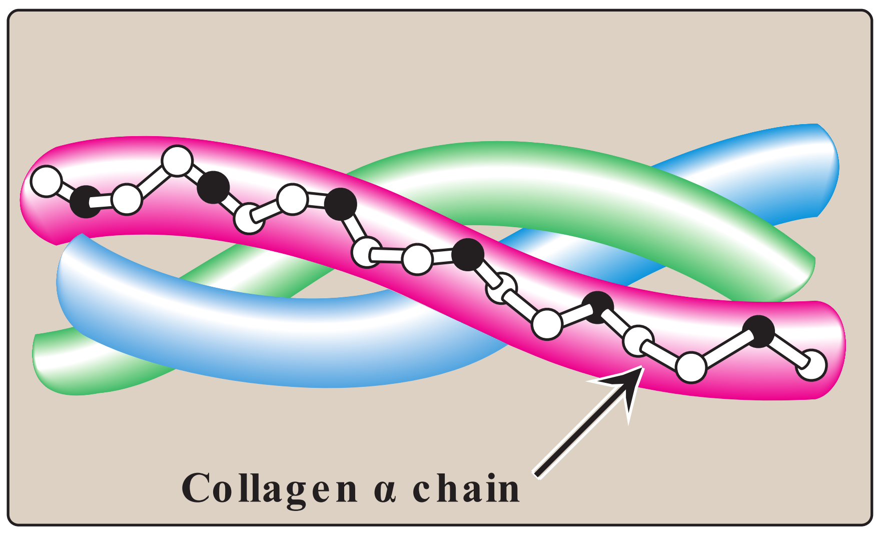


Figure 4.1
Triple-stranded helix of collagen.

A. Types

The collagen superfamily of proteins includes more than 25 collagen types as well as additional proteins that have collagen-like domains. The three polypeptide α chains are held together by interchain hydrogen bonds. Variations in the amino acid sequence of the α chains result in structural components that are about the same size (approximately 1,000 amino acids long) but with slightly different properties. These α chains are combined to form the various types of collagen found in the tissues. For example, the most common collagen, type I, contains two chains called α1 and one chain called α2 ($\alpha 1_2 \alpha 2$), whereas type II collagen contains three α1 chains ($\alpha 1_3$). The colla-

TYPE	TISSUE DISTRIBUTION
	Fibril-forming
I	Skin, bone, tendon, blood vessels, cornea
II	Cartilage, intervertebral disk, vitreous body
III	Blood vessels, skin, muscle
	Network-forming
IV	Basement membrane
VIII	Corneal and vascular endothelium
	Fibril-associated*
IX	Cartilage
XII	Tendon, ligaments, some other tissues

Figure 4.2
The most abundant types of collagen. *Known as FACITs: fibril-associated collagens with interrupted triple helices.

gens can be organized into three groups, based on their location and functions in the body (Figure 4.2).

1. **Fibril-forming collagens:** Types I, II, and III are the fibrillar collagens and have the rope-like structure described above for a typical collagen molecule. In the electron microscope, these linear polymers of fibrils have characteristic banding patterns, reflecting the regular staggered packing of the individual collagen molecules in the fibril (Figure 4.3). Type I collagen fibers (composed of collagen fibrils) are found in supporting elements of high tensile strength (for example, tendon and cornea), whereas fibers formed from type II collagen molecules are restricted to cartilaginous structures. The fibers derived from type III collagen are prevalent in more distensible tissues such as blood vessels.

2. **Network-forming collagens:** Types IV and VIII form a three-dimensional mesh, rather than distinct fibrils (Figure 4.4). For example, type IV molecules assemble into a sheet or meshwork that constitutes a major part of basement membranes.

> Basement membranes are thin, sheet-like structures that provide mechanical support for adjacent cells and function as a semipermeable filtration barrier to macromolecules in organs such as the kidney and the lung.

3. **Fibril-associated collagens:** Types IX and XII bind to the surface of collagen fibrils, linking these fibrils to one another and to other components in the extracellular matrix (see Figure 4.2).

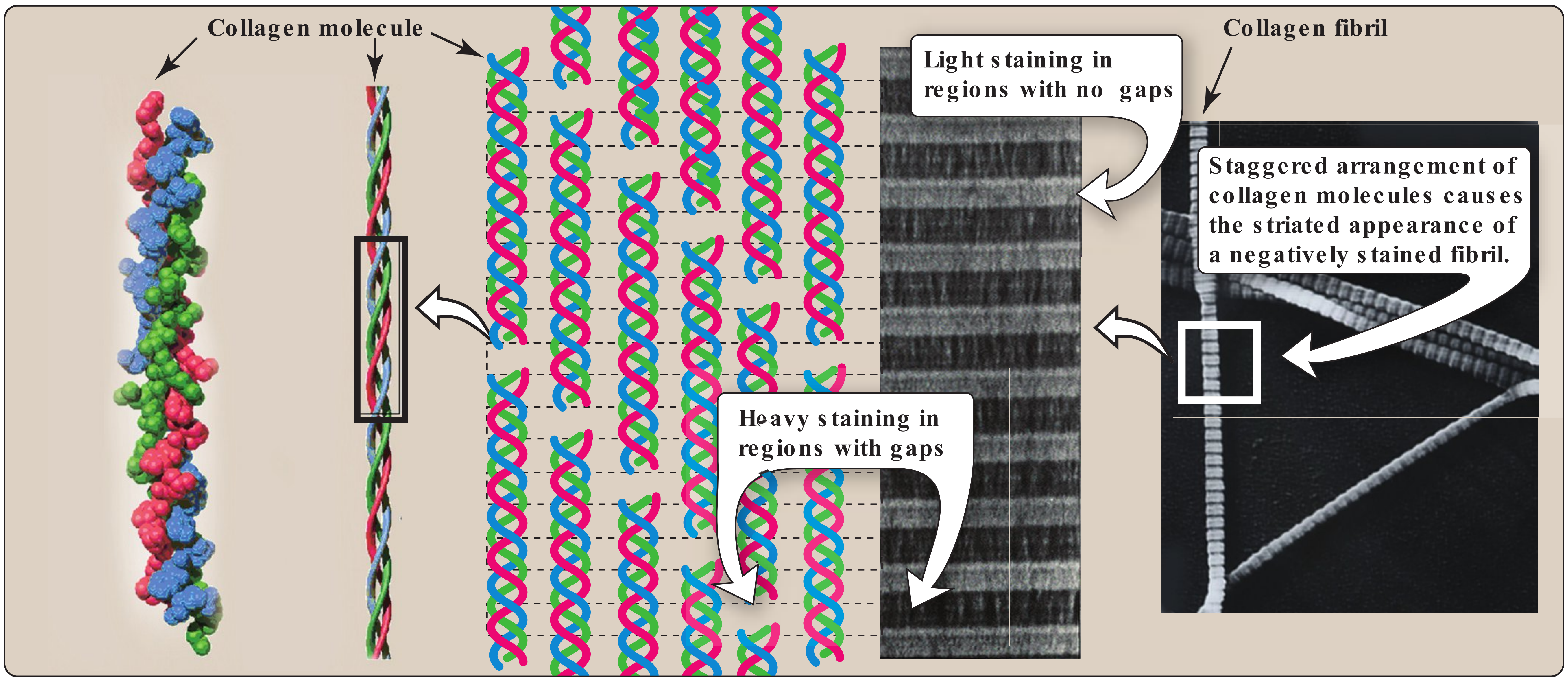

Collagen molecule
Light staining in regions with no gaps
Collagen fibril
Staggered arrangement of collagen molecules causes the striated appearance of a negatively stained fibril.
Heavy staining in regions with gaps

Figure 4.3
Collagen fibrils at right have a characteristic banding pattern, reflecting the regularly staggered packing of the individual collagen molecules in the fibril.

B. Structure

1. **Amino acid sequence:** Collagen is rich in proline and glycine, both of which are important in the formation of the triple-stranded helix. Proline facilitates the formation of the helical conformation of each α chain because its ring structure causes "kinks" in the peptide chain. [Note: The presence of proline dictates that the helical conformation of the α chain cannot be an α helix (see p. 16).] Glycine, the smallest amino acid, is found in every third position of the polypeptide chain. It fits into the restricted spaces where the three chains of the helix come together. The glycine residues are part of a repeating sequence, –Gly–X–Y–, where X is frequently proline, and Y is often hydroxyproline (but can be hydroxylysine, Figure 4.5). Thus, most of the α chain can be regarded as a polytripeptide whose sequence can be represented as $(–Gly–Pro–Hyp–)_{333}$.

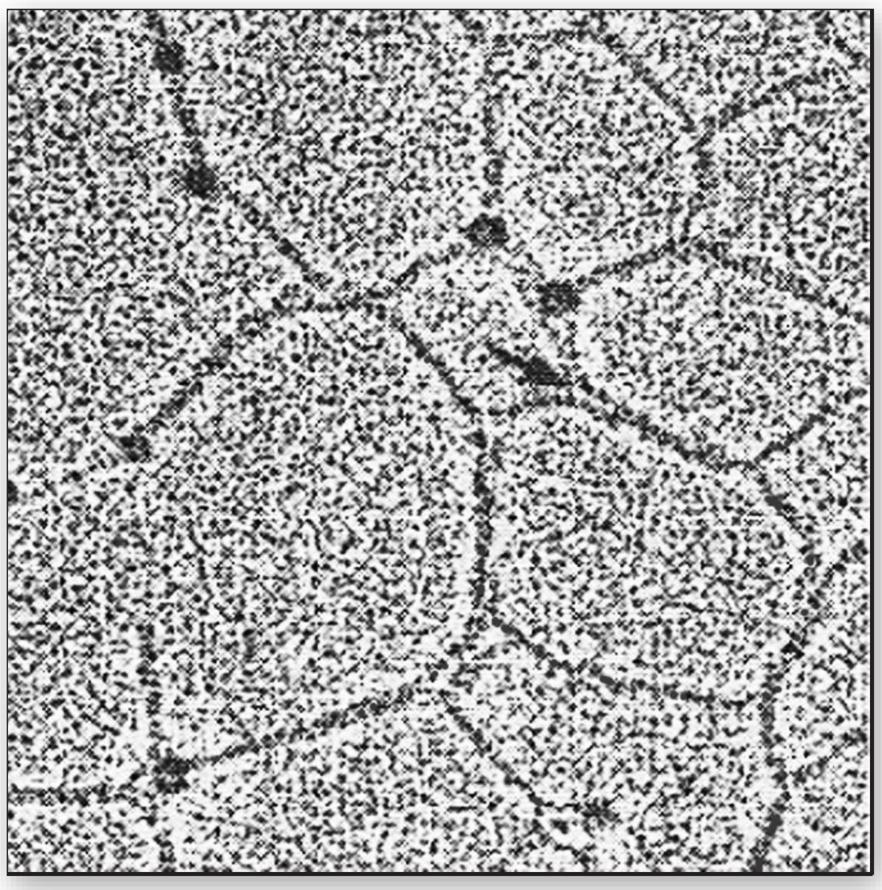


Figure 4.4
Electron micrograph of a polygonal network formed by association of collagen type IV monomers.

2. **Triple-helical structure:** Unlike most globular proteins that are folded into compact structures, collagen, a fibrous protein, has an elongated, triple-helical structure that is stabilized by interchain hydrogen bonds.

3. **Hydroxyproline and hydroxylysine:** Collagen contains hydroxyproline and hydroxylysine, which are not present in most other proteins. These residues result from the hydroxylation of some of the proline and lysine residues after their incorporation into polypeptide chains (Figure 4.6). The hydroxylation is, thus, an example of posttranslational modification (see p. 443). [Note: Generation of hydroxyproline maximizes formation of interchain hydrogen bonds that stabilize the triple-helical structure.]

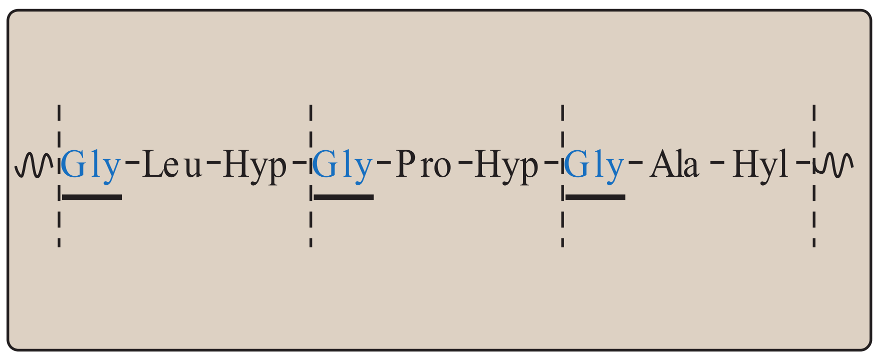


Figure 4.5
Amino acid sequence of a portion of the α1 chain of collagen. [Note: Hyp is hydroxyproline, and Hyl is hydroxylysine.]

4. **Glycosylation:** The hydroxyl group of the hydroxylysine residues of collagen may be enzymatically glycosylated. Most commonly, glucose and galactose are sequentially attached to the polypeptide chain prior to triple-helix formation (Figure 4.7).

C. Biosynthesis

The polypeptide precursors of the collagen molecule are synthesized in fibroblasts (or in the related osteoblasts of bone and chondroblasts of cartilage). They are enzymically modified and form the triple helix, which gets secreted into the extracellular matrix. After additional enzymic modification, the mature extracellular collagen monomers aggregate and become cross-linked to form collagen fibers.

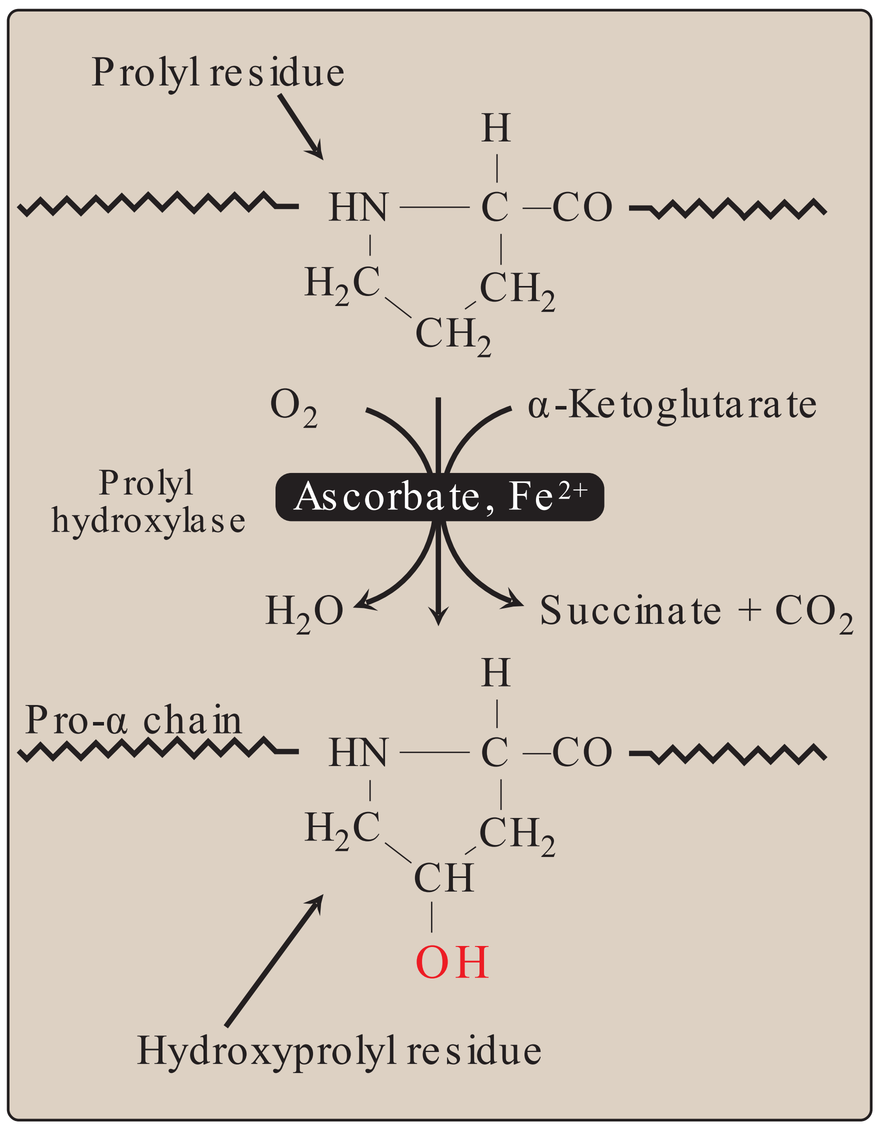
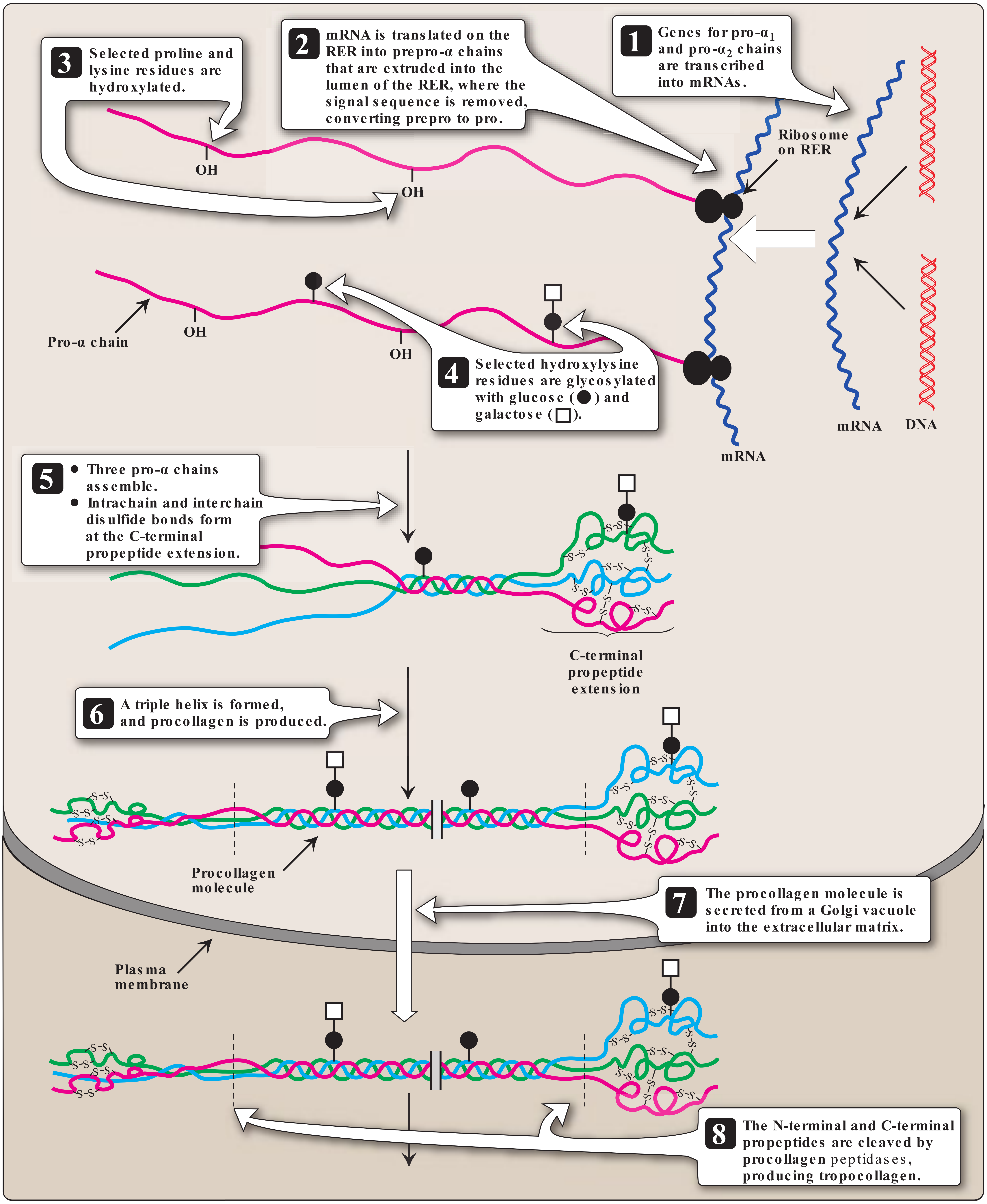


Figure 4.6
Hydroxylation of proline residues of pro-α chains of collagen by prolyl hydroxylase.

1. **Formation of pro-α chains:** Collagen is one of many proteins that normally function outside of cells. Like most proteins produced for export, the newly synthesized polypeptide precursors of α chains (prepro-α chains) contain a special amino acid sequence at their N-terminal ends. This sequence acts as a signal that, in the absence of additional signals, targets the polypeptide being synthesized for secretion from the cell. The signal sequence facilitates the binding of ribosomes to the rough endoplasmic reticulum (RER), and directs the passage of the prepro-α chain into the lumen of the RER. The signal sequence is rapidly cleaved in the RER to yield a precursor of collagen called a pro-α chain (see Figure 4.7).



1 Genes for pro-α_1 and pro-α_2 chains are transcribed into mRNAs.

2 mRNA is translated on the RER into prepro-α chains that are extruded into the lumen of the RER, where the signal sequence is removed, converting prepro to pro.

3 Selected proline and lysine residues are hydroxylated.

4 Selected hydroxylysine residues are glycosylated with glucose (●) and galactose (□).

5
- Three pro-α chains assemble.
- Intrachain and interchain disulfide bonds form at the C-terminal propeptide extension.

6 A triple helix is formed, and procollagen is produced.

7 The procollagen molecule is secreted from a Golgi vacuole into the extracellular matrix.

8 The N-terminal and C-terminal propeptides are cleaved by procollagen peptidases, producing tropocollagen.

Ribosome on RER
mRNA
DNA
mRNA
OH
OH
Pro-α chain
OH
OH
S-S
C-terminal propeptide extension
Procollagen molecule
Plasma membrane

Figure 4.7
Synthesis of collagen. RER = rough endoplasmic reticulum; mRNA = messenger RNA. (continued on the next page)

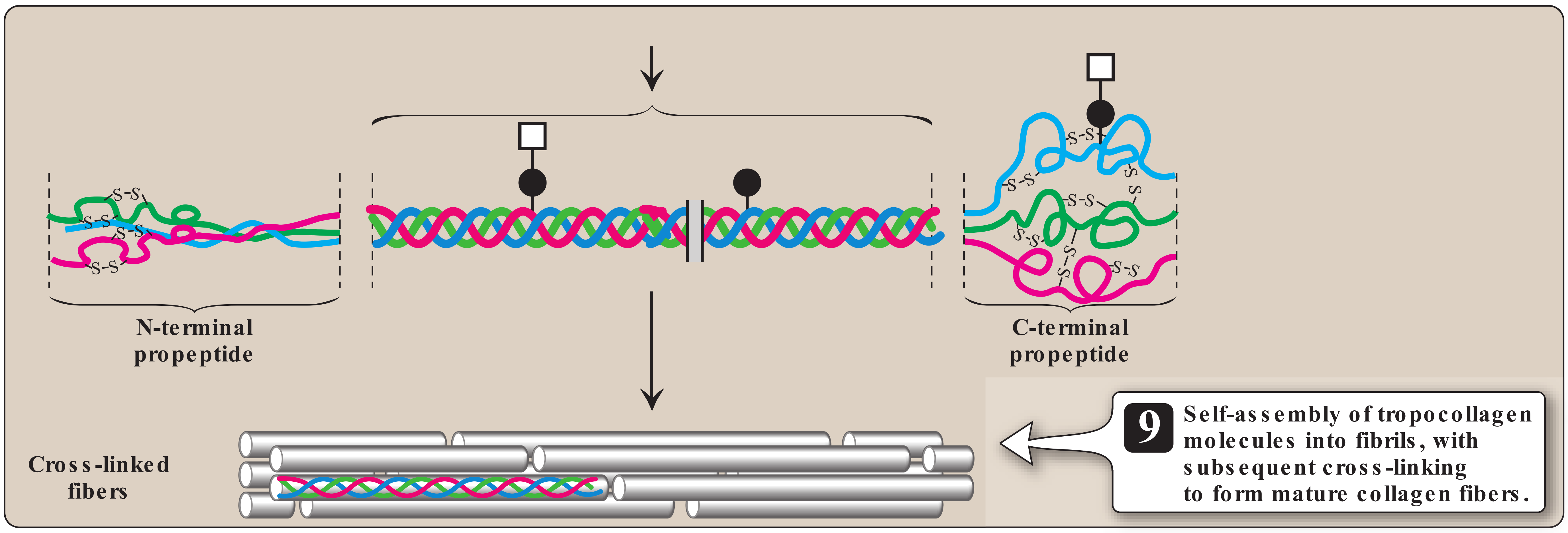

S-S
N-terminal propeptide
C-terminal propeptide
Cross-linked fibers
9 Self-assembly of tropocollagen molecules into fibrils, with subsequent cross-linking to form mature collagen fibers.

Figure 4.7
Synthesis of collagen. (continued from the previous page)

2. **Hydroxylation:** The pro-α chains are processed by a number of enzymic steps within the lumen of the RER while the polypeptides are still being synthesized (see Figure 4.7). Proline and lysine residues found in the Y-position of the –Gly–X–Y– sequence can be hydroxylated to form hydroxyproline and hydroxylysine residues. These hydroxylation reactions require molecular oxygen, Fe^{2+}, and the reducing agent vitamin C (ascorbic acid, see p. 377), without which the hydroxylating enzymes, prolyl hydroxylase and lysyl hydroxylase, are unable to function (see Figure 4.6). In the case of ascorbic acid deficiency (and, therefore, a lack of proline and lysine hydroxylation), interchain H-bond formation is impaired, as is formation of a stable triple helix. Additionally, collagen fibrils cannot be cross-linked (see below), greatly decreasing the tensile strength of the assembled fiber. The resulting deficiency disease is known as scurvy. Patients with ascorbic acid deficiency also often show bruises on the limbs as a result of subcutaneous extravasation (leakage) of blood due to capillary fragility (Figure 4.8).

3. **Glycosylation:** Some hydroxylysine residues are modified by glycosylation with glucose or glucosyl-galactose (see Figure 4.7).

4. **Assembly and secretion:** After hydroxylation and glycosylation, three pro-α chains form procollagen, a precursor of collagen that has a central region of triple helix flanked by the nonhelical amino- and carboxyl-terminal extensions called propeptides (see Figure 4.7). The formation of procollagen begins with formation of interchain disulfide bonds between the C-terminal extensions of the pro-α chains. This brings the three α chains into an alignment favorable for helix formation. The procollagen molecules move through the Golgi apparatus, where they are packaged in secretory vesicles. The vesicles fuse with the cell membrane, causing the release of procollagen molecules into the extracellular space.

5. **Extracellular cleavage of procollagen molecules:** After their release, the procollagen molecules are cleaved by N- and C-procollagen peptidases, which remove the terminal propeptides, releasing triple-helical tropocollagen molecules.

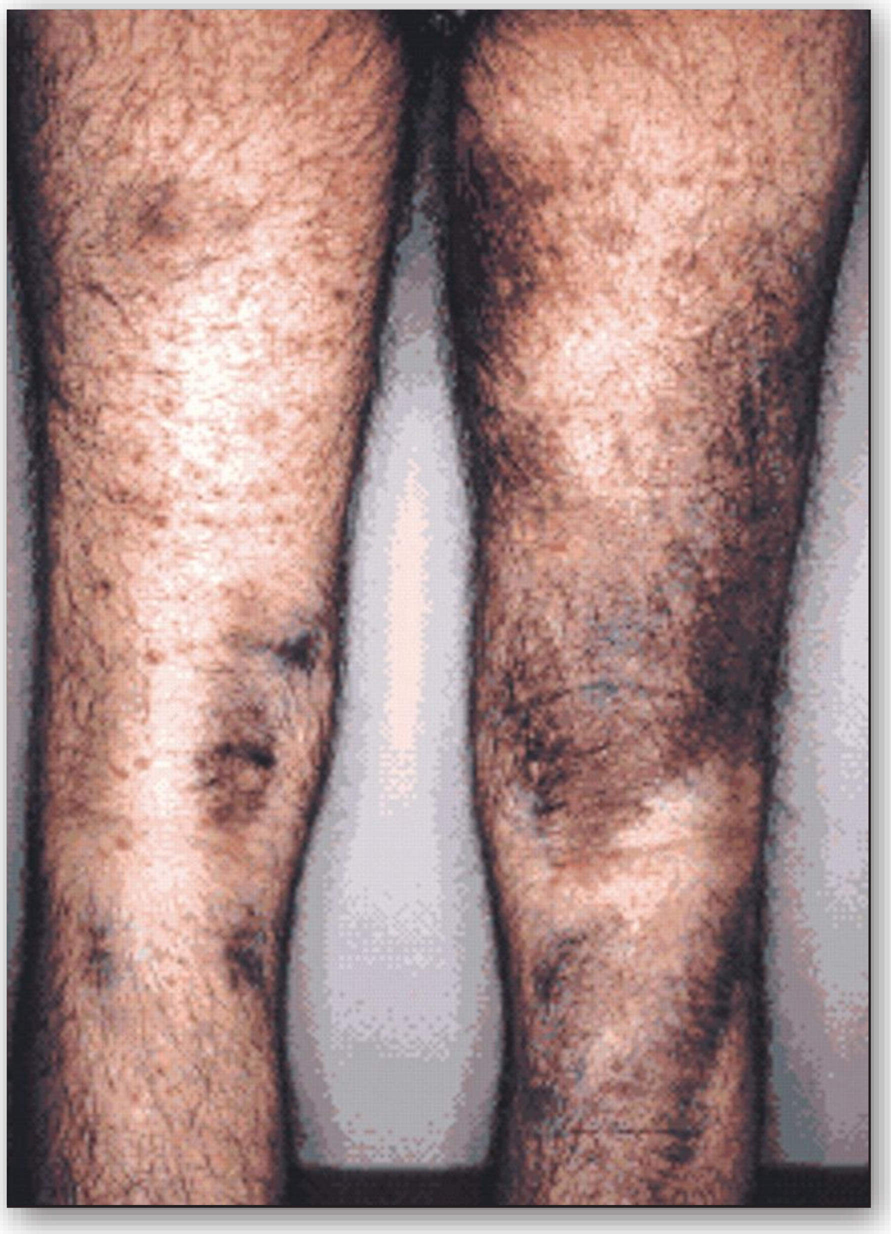


Figure 4.8
The legs of a 46-year-old man with scurvy.

6. **Formation of collagen fibrils:** Tropocollagen molecules spontaneously associate to form collagen fibrils. They form an ordered, overlapping, parallel array, with adjacent collagen molecules arranged in a staggered pattern, each overlapping its neighbor by a length approximately three-quarters of a molecule (see Figure 4.7).

7. **Cross-link formation:** The fibrillar array of collagen molecules serves as a substrate for lysyl oxidase. This Cu^{2+}-containing extracellular enzyme oxidatively deaminates some of the lysine and hydroxylysine residues in collagen. The reactive aldehydes that result (allysine and hydroxyallysine) can condense with lysine or hydroxylysine residues in neighboring collagen molecules to form covalent cross-links and, thus, mature collagen fibers (Figure 4.9).

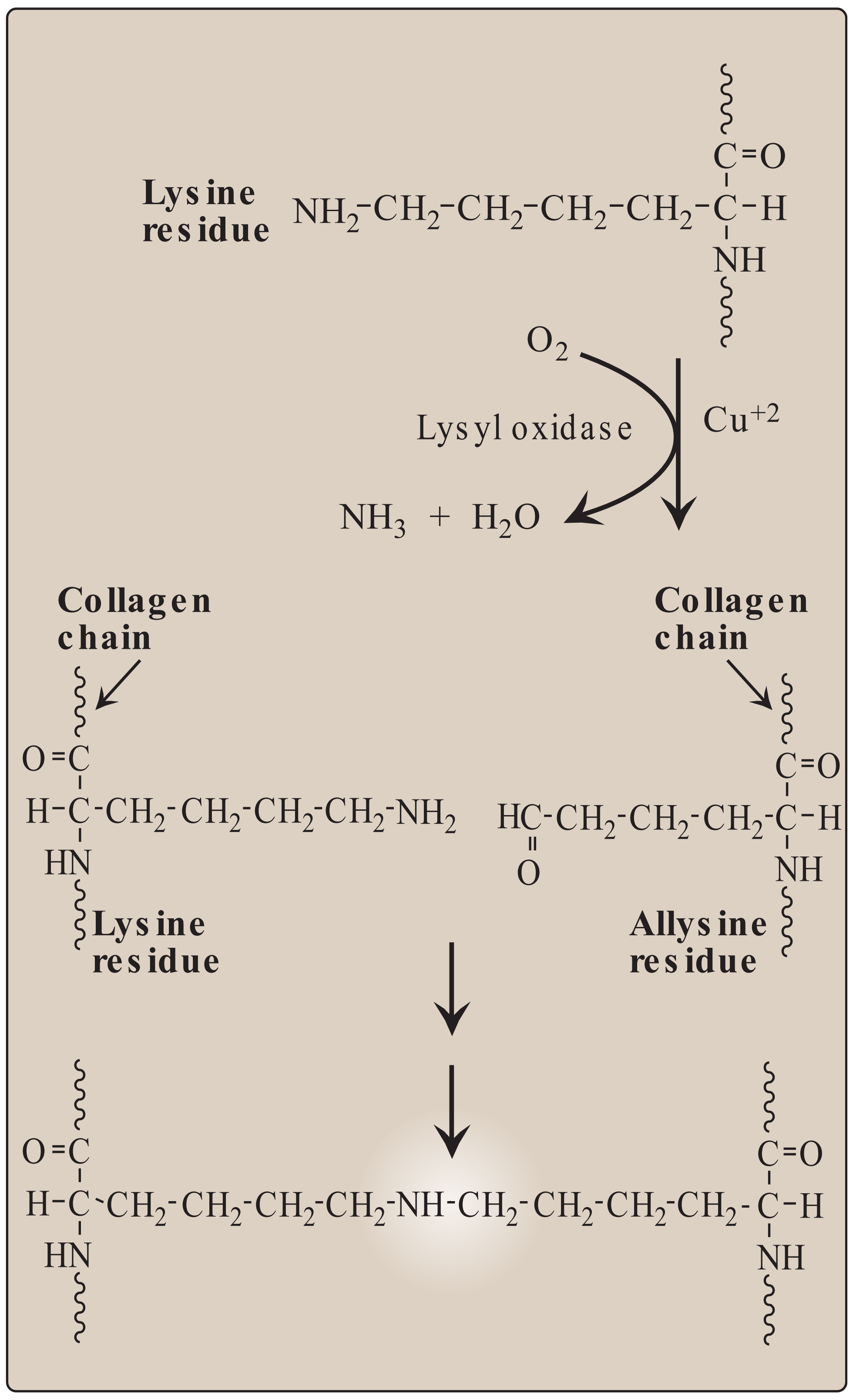

Lysine residue
$NH_2-CH_2-CH_2-CH_2-CH_2-C-H$
C=O
NH
O_2
Lysyl oxidase
Cu^{+2}
$NH_3 + H_2O$
Collagen chain
Collagen chain
O=C
H-C-CH_2-CH_2-CH_2-CH_2-NH_2
HN
Lysine residue
HC-CH_2-CH_2-CH_2-C-H
O
C=O
NH
Allysine residue
O=C
H-C-CH_2-CH_2-CH_2-CH_2-NH-CH_2-CH_2-CH_2-CH_2-C-H
HN
C=O
NH

Figure 4.9
Formation of cross-links in collagen. [Note: Lysyl oxidase is irreversibly inhibited by a toxin from plants in the genus Lathyrus, leading to a condition known as lathyrism.]

Lysyl oxidase is one of several copper-containing enzymes. Others include cytochrome oxidase (see p. 76), dopamine hydroxylase (see p. 286), superoxide dismutase (see p.148), and tyrosinase (see p. 273). Disruption in copper homeostasis causes copper deficiency (X-linked Menkes disease) or overload (Wilson disease).

D. Degradation

Normal collagens are highly stable molecules, having half-lives as long as several years. However, connective tissue is dynamic and is constantly being remodeled, often in response to growth or injury of the tissue. Breakdown of collagen fibers is dependent on the proteolytic action of collagenases, which are part of a large family of matrix metalloproteinases. For type I collagen, the cleavage site is specific, generating three-quarter and one-quarter length fragments. These fragments are further degraded by other matrix proteinases.

E. Collagen diseases: Collagenopathies

Defects in any one of the many steps in collagen fiber synthesis can result in a genetic disease involving an inability of collagen to form fibers properly and, therefore, an inability to provide tissues with the needed tensile strength normally provided by collagen. More than 1,000 mutations have been identified in 23 genes coding for 13 of the collagen types. The following are examples of diseases that are the result of defective collagen synthesis.

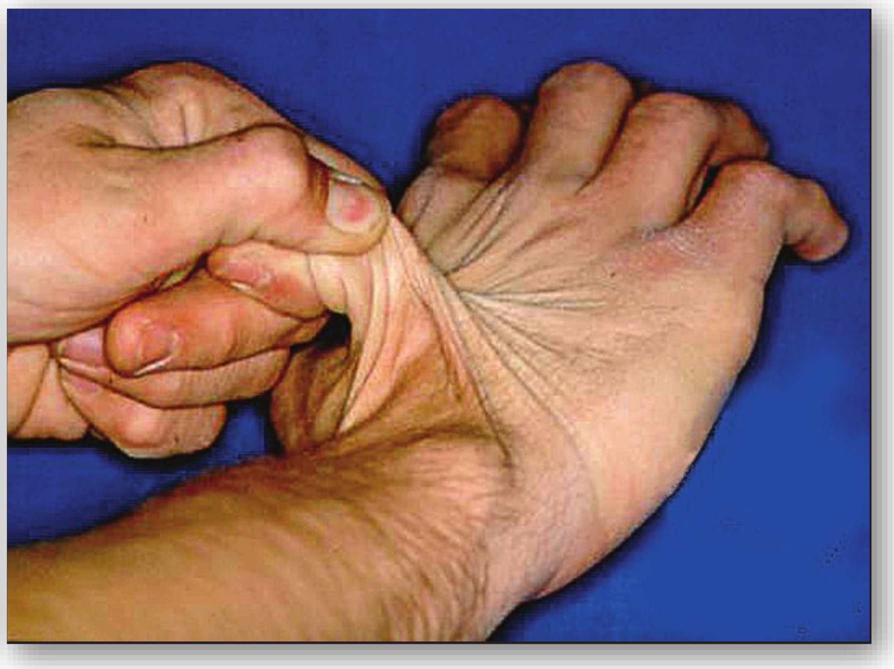


Figure 4.10
Stretchy skin of classic Ehlers-Danlos syndrome.

1. **Ehlers-Danlos syndrome:** Ehlers-Danlos syndrome (EDS) is a heterogeneous group of connective tissue disorders that result from inheritable defects in the metabolism of fibrillar collagen molecules. EDS can be caused by a deficiency of collagen-processing enzymes (for example, lysyl hydroxylase or N-procollagen peptidase) or from mutations in the amino acid sequences of collagen types I, III, or V. The classic form of EDS, caused by defects in type V collagen, is characterized by skin extensibility and fragility and joint hypermobility (Figure 4.10). The vascular form, due to defects in type III collagen, is the most serious form of EDS because it is associated with potentially lethal arterial rupture. [Note: The classic and vascular forms show autosomal dominant

inheritance.] Collagen containing mutant chains may have altered structure, secretion, or distribution. It frequently is degraded. [Note: Incorporation of just one mutant chain may result in degradation of the triple helix. This is known as a dominant-negative effect.].

2. **Osteogenesis imperfecta:** This syndrome, known as brittle bone disease, is a genetic disorder of bone fragility characterized by bones that fracture easily, with minor or no trauma (Figure 4.11). Over 80% of cases of osteogenesis imperfecta (OI) are caused by dominant mutations to the genes that code for the α1 or α2 chains in type I collagen. The most common mutations cause the replacement of glycine (in –Gly–X–Y–) by amino acids with bulky side chains. The resultant structurally abnormal α chains prevent the formation of the required triple-helical conformation. Phenotypic severity ranges from mild to lethal. Type I OI, the most common form, is characterized by mild bone fragility, hearing loss, and blue sclerae. Type II, the most severe form, is typically lethal in the perinatal period as a result of pulmonary complications. In utero fractures are seen (see Figure 4.11). Type III is also a severe form. It is characterized by multiple fractures at birth, short stature, spinal curvature leading to a "humped-back" (kyphotic) appearance, and blue sclerae. Dentinogenesis imperfecta, a disorder of tooth development, may be seen in OI.

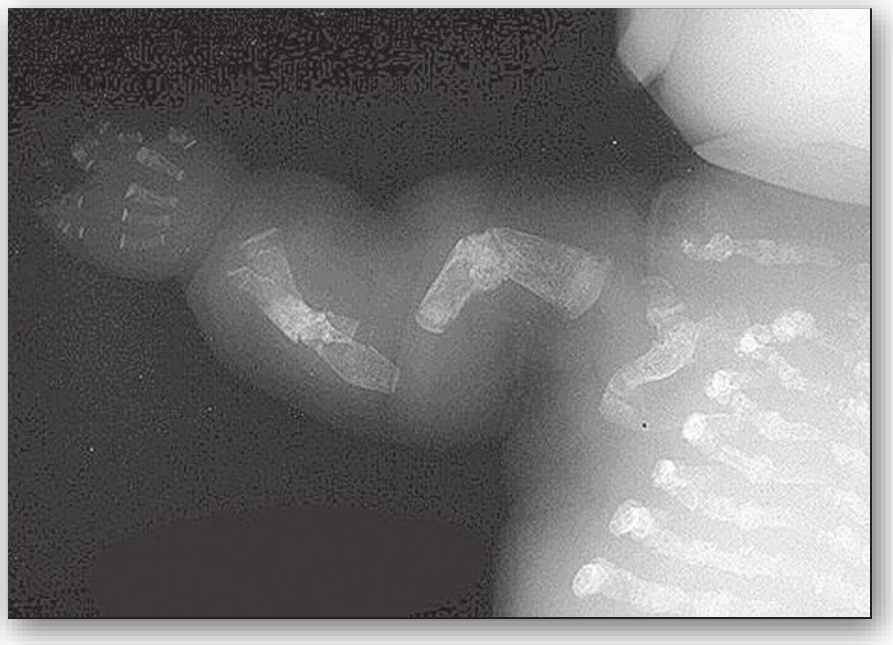


Figure 4.11
Lethal form (type II) of osteogenesis imperfecta in which the fractures appear in utero, as revealed by this radiograph of a stillborn fetus.

III. ELASTIN

In contrast to collagen, which forms fibers that are tough and have high tensile strength, elastin is a connective tissue protein with rubber-like properties. Elastic fibers composed of elastin and glycoprotein microfibrils are found in the lungs, the walls of large arteries, and elastic ligaments. They can be stretched to several times their normal length but recoil to their original shape when the stretching force is relaxed.

A. Structure

Elastin is an insoluble protein polymer synthesized from a precursor, tropoelastin, which is a linear polypeptide composed of about 700 amino acids that are primarily small and nonpolar (for example, glycine, alanine, and valine). Elastin is also rich in proline and lysine but contains scant hydroxyproline and hydroxylysine. Tropoelastin is secreted by the cell into the extracellular space. There, it interacts with specific glycoprotein microfibrils, such as fibrillin, which function as a scaffold onto which tropoelastin is deposited. Some of the lysyl side chains of the tropoelastin polypeptides are oxidatively deaminated by lysyl oxidase, forming allysine residues. Three of the allysyl side chains plus one unaltered lysyl side chain from the same or neighboring polypeptides form a desmosine cross-link (Figure 4.12). This produces elastin, an extensively interconnected, rubbery network that can stretch and bend in any direction when stressed, giving connective tissue elasticity (Figure 4.13). Mutations in the fibrillin-1 protein are responsible for Marfan syndrome, a connective tissue disorder characterized by impaired structural integrity in the skeleton, the eye, and the cardiovascular system. With this disease, abnormal fibrillin protein is incorporated into microfibrils along with normal fibrillin, inhibiting the formation of functional microfibrils. [Note: Patients with Marfan syndrome, OI, or EDS may have blue sclerae due to tissue thinning that allows underlying pigment to show through.]

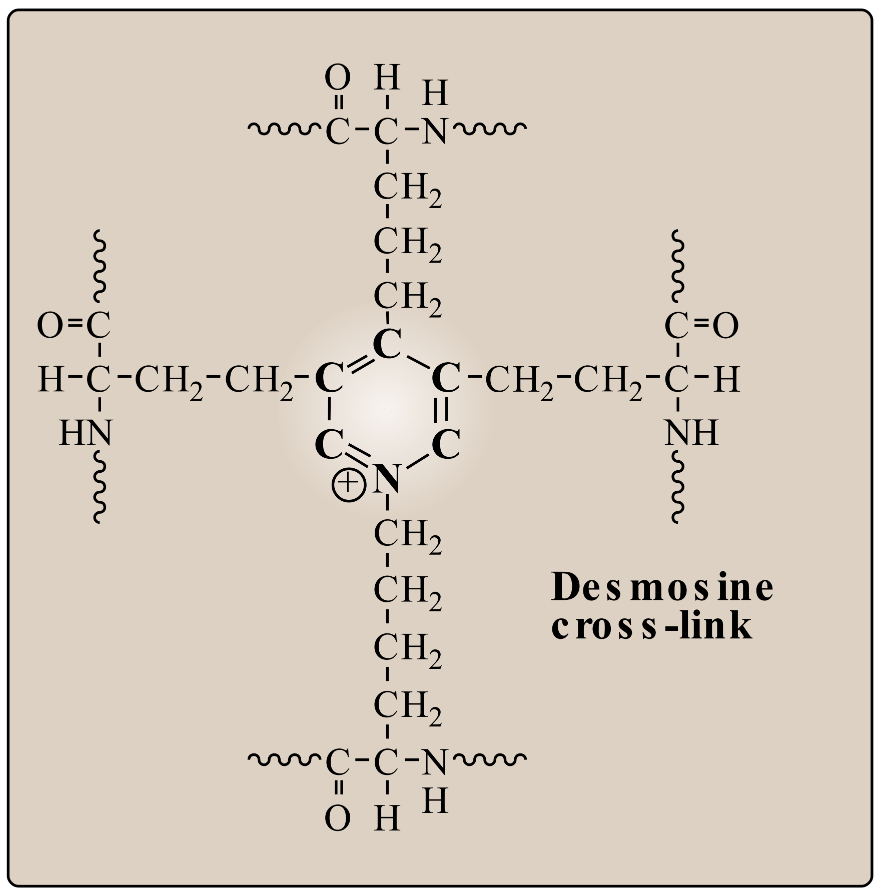


Figure 4.12
Desmosine cross-link in elastin.

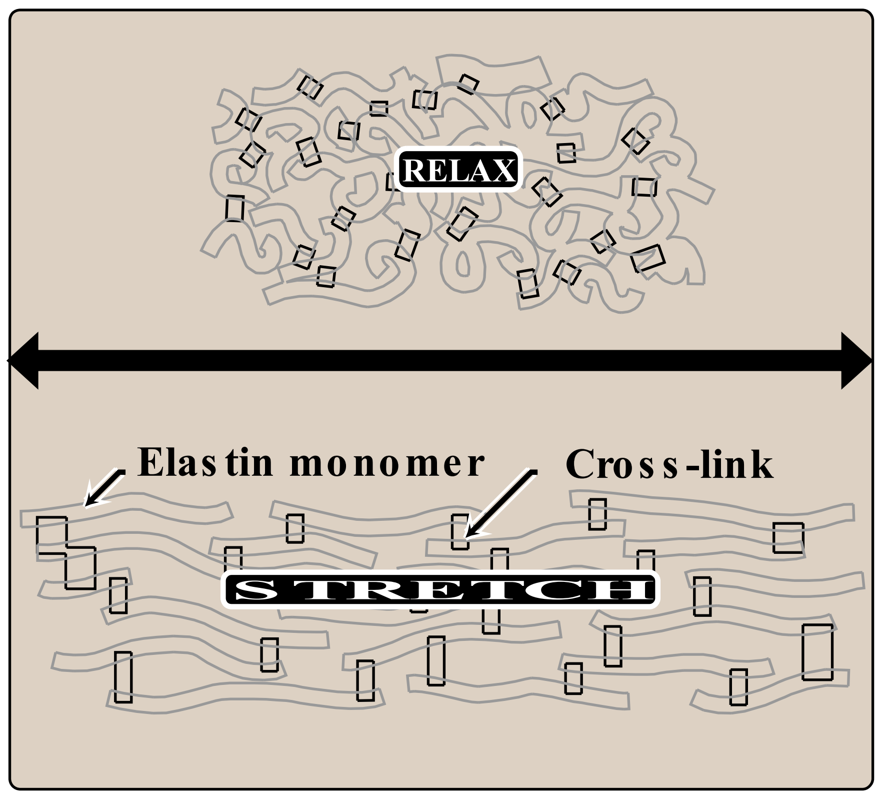


Figure 4.13
Elastin fibers in relaxed and stretched conformations.

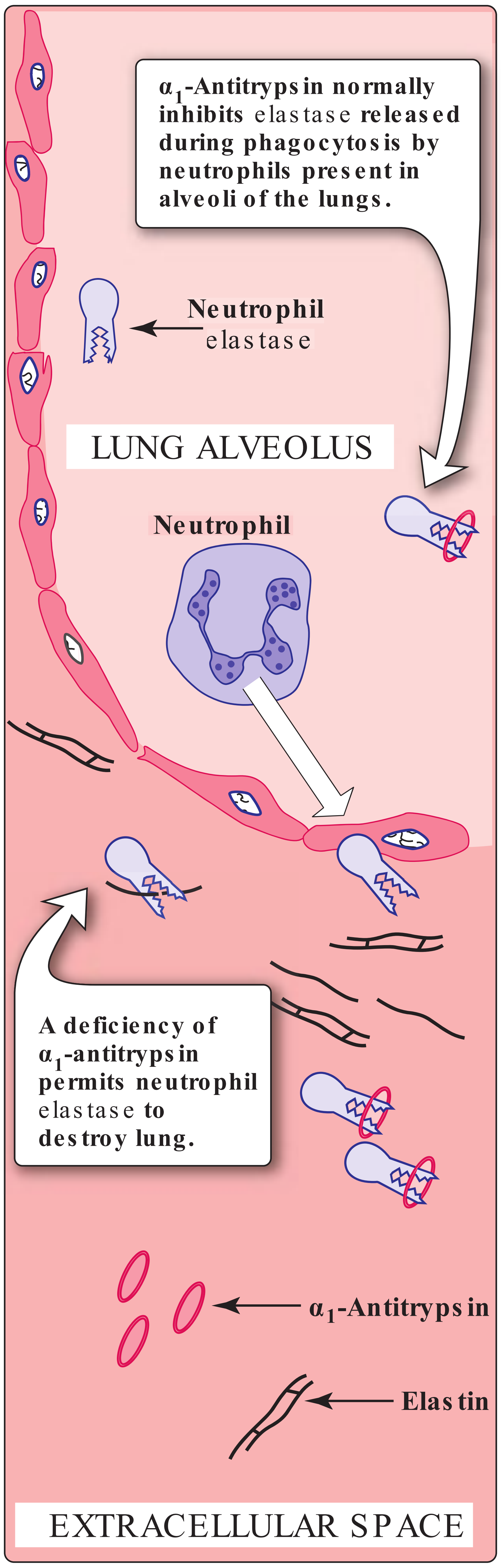

α_1-Antitrypsin normally inhibits elastase released during phagocytosis by neutrophils present in alveoli of the lungs.
Neutrophil elastase
LUNG ALVEOLUS
Neutrophil
A deficiency of α_1-antitrypsin permits neutrophil elastase to destroy lung.
α_1-Antitrypsin
Elastin
EXTRACELLULAR SPACE

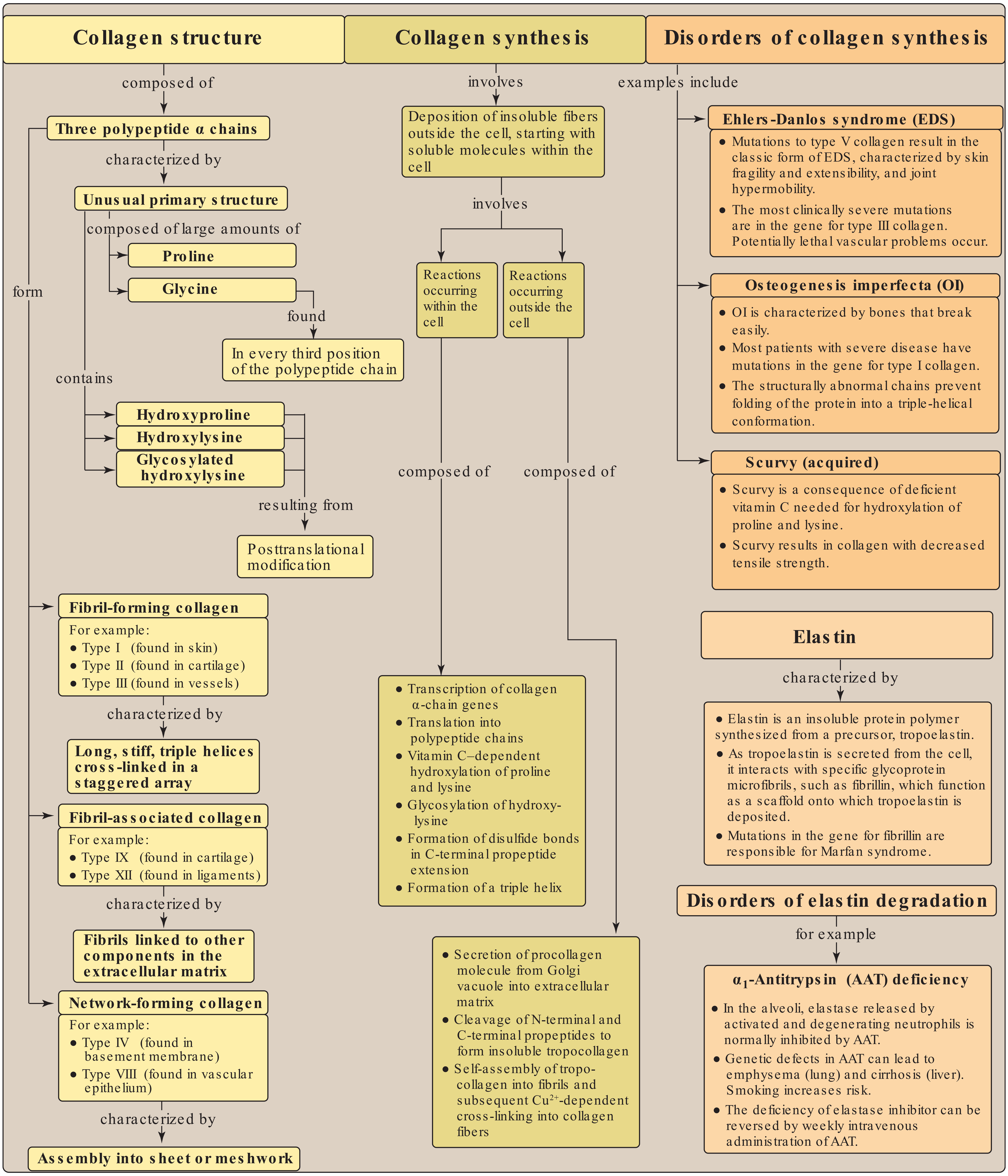
Figure 4.14
Destruction of alveolar tissue by elastase released from neutrophils activated as part of the immune response to airborne pathogens.

B. Role of α_1-antitrypsin in elastin degradation

1. **α_1-Antitrypsin:** Blood and other body fluids contain a protein, α_1-antitrypsin (AAT or A1AT), which inhibits a number of proteolytic enzymes (called proteases or proteinases) that hydrolyze and destroy proteins. [Note: The inhibitor was originally named α1-antitrypsin because it inhibits the activity of trypsin, a proteolytic enzyme synthesized as trypsinogen by the pancreas (see p. 248).] AAT has the important physiologic role of inhibiting neutrophil elastase, a powerful protease that is released into the extracellular space and degrades elastin of alveolar walls as well as other structural proteins in a variety of tissues (Figure 4.14). Most of the AAT found in plasma is synthesized and secreted by the liver. AAT comprises more than 90% of the α_1-globulin fraction of normal plasma. Extrahepatic synthesis occurs in monocytes and alveolar macrophages, and may be important in the prevention of local tissue injury by elastase.

2. **Role of α_1-antitrypsin in the lungs:** In the normal lung, the alveoli are chronically exposed to low levels of neutrophil elastase released from activated and degenerating neutrophils. The proteolytic activity of elastase can destroy the elastin in alveolar walls if unopposed by the action of AAT, the most important inhibitor of neutrophil elastase (see Figure 4.14). Because lung tissue cannot regenerate, the destruction of the connective tissue of alveolar walls results in emphysema.

3. **Emphysema resulting from α_1-antitrypsin deficiency:** In the United States, approximately 2%–5% of patients with emphysema are predisposed to the disease by inherited defects in AAT. A number of different mutations in the gene for AAT are known to cause a deficiency of the protein, but one single purine base mutation (GAG to AAG, resulting in the substitution of lysine for glutamic acid at position 342 of the protein) is clinically the most widespread. The mutation causes the normally monomeric AAT to polymerize within the endoplasmic reticulum of hepatocytes, resulting in decreased secretion of AAT by the liver. Consequently, blood levels of AAT are reduced, decreasing the amount that gets to the alveoli. The polymer that accumulates in the liver may result in cirrhosis (scarring of the liver). In the United States, the AAT mutation is most common in Caucasians of Northern European ancestry. An individual must inherit two abnormal AAT alleles to be at risk for the development of emphysema. In a heterozygote, with one normal and one defective gene, the levels of AAT are sufficient to protect the alveoli from damage. [Note: Methionine 358 in AAT is required for the binding of the inhibitor to its target proteases. Smoking causes the oxidation and subsequent inactivation of the methionine, thereby rendering the inhibitor powerless to neutralize elastase. Smokers with AAT deficiency, therefore, have a considerably elevated rate of lung destruction and a poorer survival rate than nonsmokers with the deficiency.] The deficiency of elastase inhibitor can be treated by weekly augmentation therapy, that is, intravenous administration of AAT. The AAT diffuses from the blood into the lung, where it reaches therapeutic levels in the fluid surrounding the lung epithelial cells.



Collagen structure

Three polypeptide α chains

Unusual primary structure

Proline

Glycine

In every third position of the polypeptide chain

Hydroxyproline

Hydroxylysine

Glycosylated hydroxylysine

Posttranslational modification

Fibril-forming collagen

For example:

- Type I (found in skin)
- Type II (found in cartilage)
- Type III (found in vessels)

Long, stiff, triple helices cross-linked in a staggered array

Fibril-associated collagen

For example:

- Type IX (found in cartilage)
- Type XII (found in ligaments)

Fibrils linked to other components in the extracellular matrix

Network-forming collagen

For example:

- Type IV (found in basement membrane)
- Type VIII (found in vascular epithelium)

Assembly into sheet or meshwork

Collagen synthesis

Deposition of insoluble fibers outside the cell, starting with soluble molecules within the cell

Reactions occurring within the cell

Reactions occurring outside the cell

- Transcription of collagen α-chain genes
- Translation into polypeptide chains
- Vitamin C–dependent hydroxylation of proline and lysine
- Glycosylation of hydroxylysine
- Formation of disulfide bonds in C-terminal propeptide extension
- Formation of a triple helix

- Secretion of procollagen molecule from Golgi vacuole into extracellular matrix
- Cleavage of N-terminal and C-terminal propeptides to form insoluble tropocollagen
- Self-assembly of tropocollagen into fibrils and subsequent Cu^{2+}-dependent cross-linking into collagen fibers

Disorders of collagen synthesis

Ehlers-Danlos syndrome (EDS)

- Mutations to type V collagen result in the classic form of EDS, characterized by skin fragility and extensibility, and joint hypermobility.
- The most clinically severe mutations are in the gene for type III collagen. Potentially lethal vascular problems occur.

Osteogenesis imperfecta (OI)

- OI is characterized by bones that break easily.
- Most patients with severe disease have mutations in the gene for type I collagen.
- The structurally abnormal chains prevent folding of the protein into a triple-helical conformation.

Scurvy (acquired)

- Scurvy is a consequence of deficient vitamin C needed for hydroxylation of proline and lysine.
- Scurvy results in collagen with decreased tensile strength.

Elastin

- Elastin is an insoluble protein polymer synthesized from a precursor, tropoelastin.
- As tropoelastin is secreted from the cell, it interacts with specific glycoprotein microfibrils, such as fibrillin, which function as a scaffold onto which tropoelastin is deposited.
- Mutations in the gene for fibrillin are responsible for Marfan syndrome.

Disorders of elastin degradation

α_1-Antitrypsin (AAT) deficiency

- In the alveoli, elastase released by activated and degenerating neutrophils is normally inhibited by AAT.
- Genetic defects in AAT can lead to emphysema (lung) and cirrhosis (liver). Smoking increases risk.
- The deficiency of elastase inhibitor can be reversed by weekly intravenous administration of AAT.

Figure 4.15
Key concept map for the fibrous proteins collagen and elastin.

IV. CHAPTER SUMMARY

Collagen and elastin are fibrous proteins (Figure 4.15). Collagen molecules contain an abundance of **proline**, **lysine**, and **glycine**, the latter occurring at every third position in the primary structure. Collagen also contains **hydroxyproline**, **hydroxylysine**, and **glycosylated hydroxylysine**, each formed by posttranslational modification. Collagen molecules typically form **fibrils** containing a long, stiff, triple-stranded helical structure, in which three collagen polypeptide chains are wound around one another in a rope-like superhelix (**triple helix**). Other types of collagen form mesh-like networks. **Elastin** is a connective tissue protein with rubber-like properties in tissues such as the lung. **α_1-Antitrypsin** (**AAT**), produced primarily by the liver but also by monocytes and alveolar macrophages, prevents elastase-catalyzed degradation of elastin in the alveolar walls. A deficiency of AAT can cause **emphysema** and, in some cases, cirrhosis of the liver.

Enzymes

5

I. OVERVIEW

Virtually all reactions in the body are mediated by enzymes, which are protein catalysts that increase the rate of reactions without being changed in the overall process. Among the many biologic reactions that are energetically possible, enzymes selectively channel reactants (called substrates) into useful pathways. Enzymes thus direct all metabolic events. This chapter examines the nature of these catalytic molecules and their mechanism of action.

II. NOMENCLATURE

Each enzyme is assigned two names. The first is its short, recommended name, convenient for everyday use. The second is the more complete systematic name, which is used when an enzyme must be identified without ambiguity.

A. Recommended name

Most commonly used enzyme names have the suffix “-ase” attached to the substrate of the reaction (for example, glucosidase and urease) or to a description of the action performed (for example, lactate dehydrogenase and adenylyl cyclase). [Note: Some enzymes retain their original trivial names, which give no hint of the associated enzymic reaction, for example, trypsin and pepsin.]

B. Systematic name

In the systematic naming system, enzymes are divided into six major classes (Figure 5.1), each with numerous subgroups. For a given enzyme, the suffix -ase is attached to a fairly complete description of the chemical reaction catalyzed, including the names of all the substrates, for example, lactate:NAD$^+$ oxidoreductase. [Note: Each enzyme is also assigned a classification number. Lactate:NAD$^+$ oxidoreductase, for example, is 1.1.1.27.] The systematic names are unambiguous and informative but are frequently too cumbersome to be of general use.

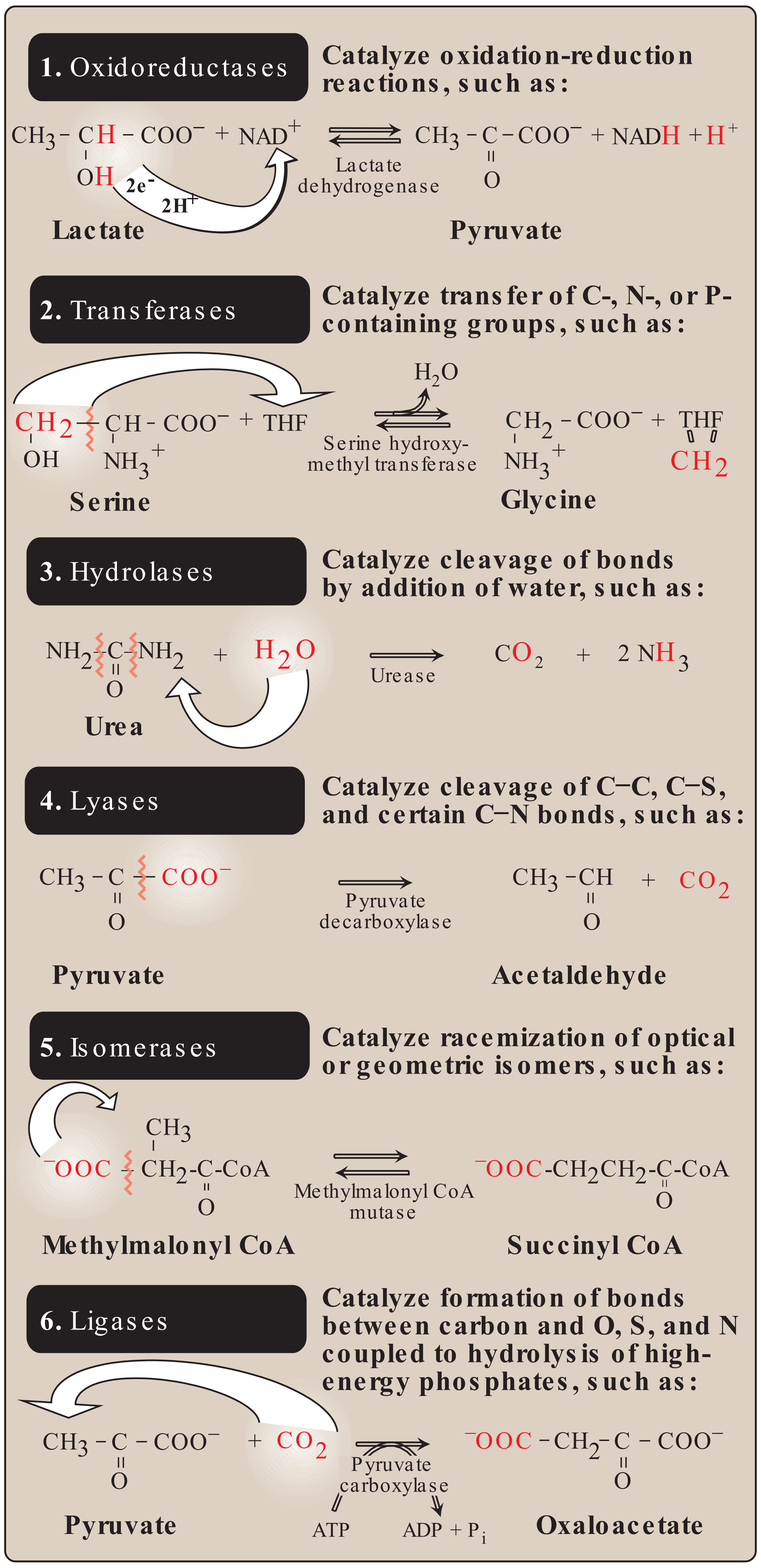

1. Oxidoreductases
Catalyze oxidation-reduction reactions, such as:
CH3-CH-COO⁻ + NAD⁺
OH
2e⁻
2H⁺
Lactate dehydrogenase
CH3-C-COO⁻ + NADH + H⁺
O
Lactate
Pyruvate
2. Transferases
Catalyze transfer of C-, N-, or P-containing groups, such as:
CH2-CH-COO⁻ + THF
OH
NH3⁺
H2O
Serine hydroxymethyl transferase
CH2-COO⁻ + THF
NH3⁺
CH2
Serine
Glycine
3. Hydrolases
Catalyze cleavage of bonds by addition of water, such as:
NH2-C-NH2 + H2O
O
Urease
CO2 + 2 NH3
Urea
4. Lyases
Catalyze cleavage of C–C, C–S, and certain C–N bonds, such as:
CH3-C-COO⁻
O
Pyruvate decarboxylase
CH3-CH + CO2
O
Pyruvate
Acetaldehyde
5. Isomerases
Catalyze racemization of optical or geometric isomers, such as:
CH3
⁻OOC-CH2-C-CoA
O
Methylmalonyl CoA mutase
⁻OOC-CH2CH2-C-CoA
O
Methylmalonyl CoA
Succinyl CoA
6. Ligases
Catalyze formation of bonds between carbon and O, S, and N coupled to hydrolysis of high-energy phosphates, such as:
CH3-C-COO⁻ + CO2
O
Pyruvate carboxylase
ATP
ADP + Pi
⁻OOC-CH2-C-COO⁻
O
Pyruvate
Oxaloacetate

Figure 5.1
The six major classes of enzymes with examples. NAD(H) = nicotinamide adenine dinucleotide; THF = tetrahydrofolate; CoA = coenzyme A.

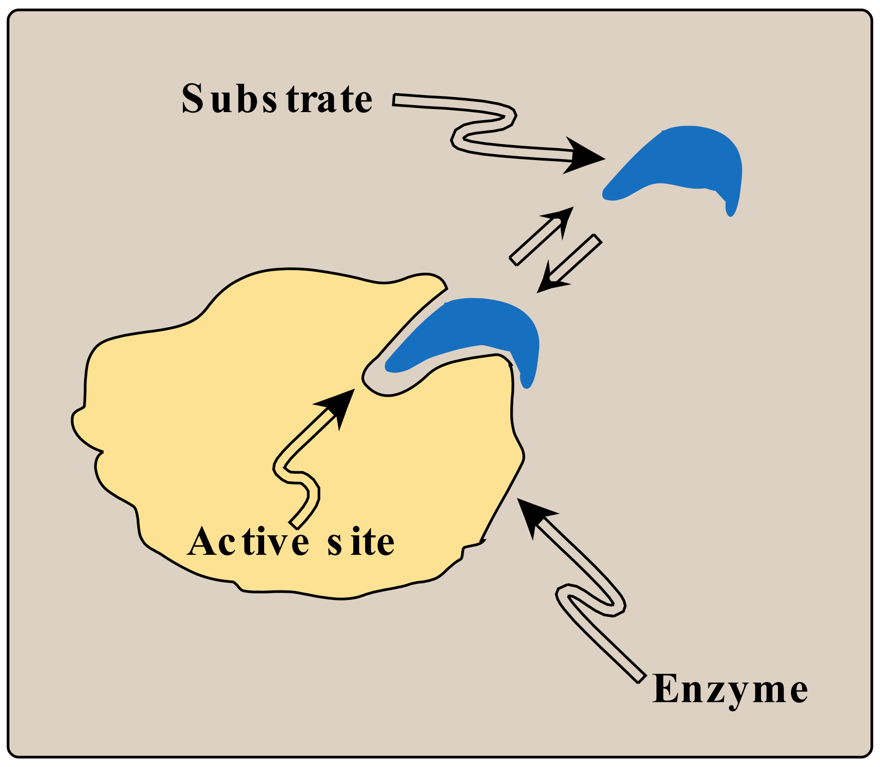


Figure 5.2
Schematic representation of an enzyme with one active site binding a substrate molecule.

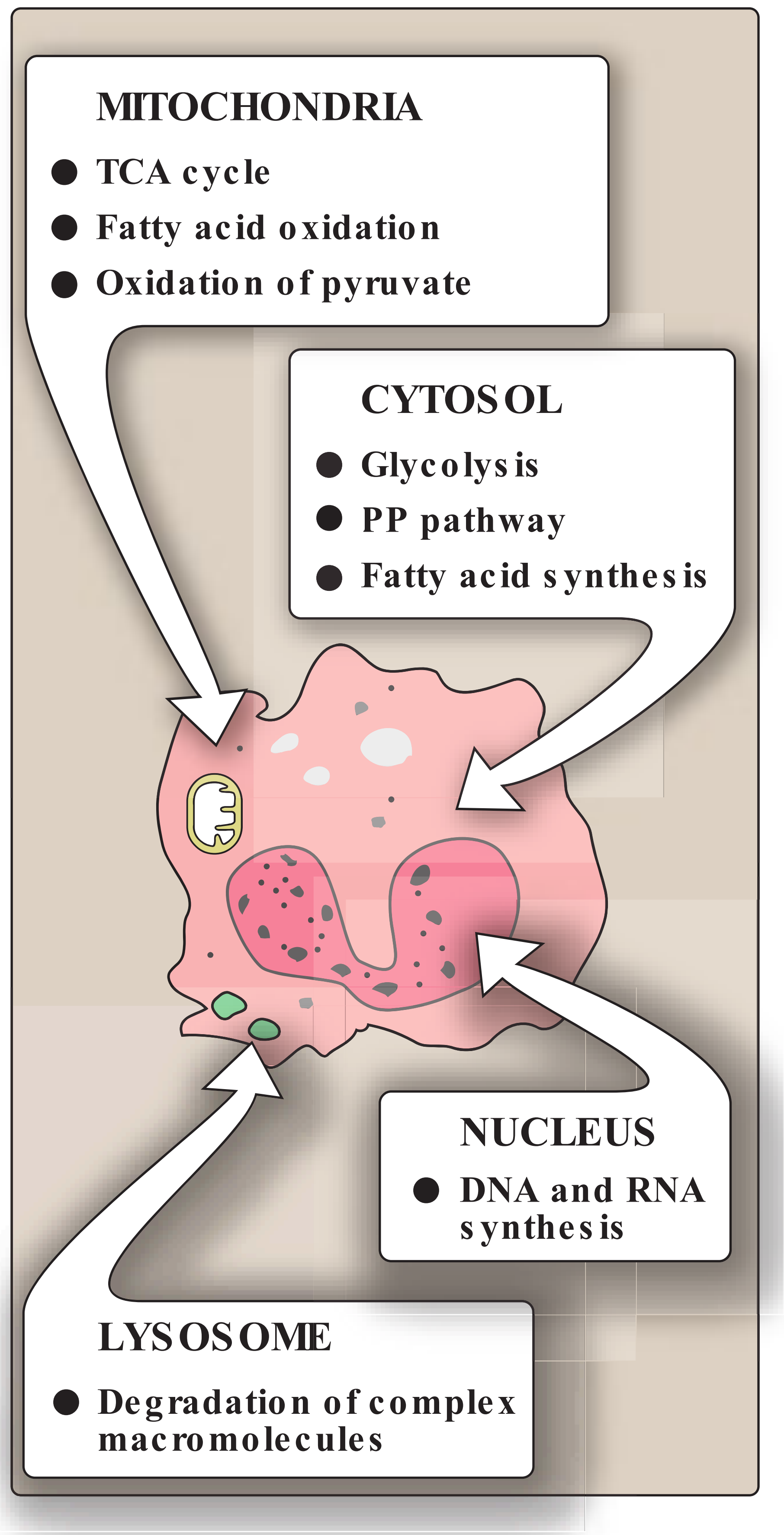


Figure 5.3
The intracellular location of some important biochemical pathways. TCA = tricarboxylic acid; PP = pentose phosphate.

> Potentially confusing enzyme nomenclature: synthetase (requires ATP), synthase (no ATP required); phosphatase (uses water to remove phosphoryl group), phosphorylase (uses P_i to break a bond and generate a phosphorylated product); dehydrogenase (NAD^+/FAD is an electron acceptor in a redox reaction), oxidase (O_2 is the acceptor, and oxygen atoms are not incorporated into substrate), oxygenase (one or both oxygen atoms are incorporated).

III. PROPERTIES

Enzymes are protein catalysts that increase the velocity of a chemical reaction and are not consumed during the reaction. [Note: Some RNAs can act like enzymes, usually catalyzing the cleavage and synthesis of phosphodiester bonds. RNAs with catalytic activity are called ribozymes (see p. 439) and are much less commonly encountered than protein catalysts.]

A. Active sites

Enzyme molecules contain a special pocket or cleft called the active site. The active site, formed by folding of the protein, contains amino acid side chains that participate in substrate binding and catalysis (Figure 5.2). The substrate binds the enzyme, forming an enzyme–substrate (ES) complex. Binding is thought to cause a conformational change in the enzyme (induced fit model) that allows catalysis. ES is converted to an enzyme–product (EP) complex that subsequently dissociates to enzyme and product.

B. Catalytic efficiency

Enzyme-catalyzed reactions are highly efficient, proceeding from 10^3–10^8 times faster than uncatalyzed reactions. The number of molecules of substrate converted to product per enzyme molecule per second is called the turnover number, or k_{cat}, and typically is 10^2–$10^4 s^{-1}$.

C. Specificity

Enzymes are highly specific, interacting with one or a few substrates and catalyzing only one type of chemical reaction. The set of enzymes made in a cell determines which reactions occur in that cell.

D. Holoenzymes, apoenzymes, cofactors, and coenzymes

Some enzymes require molecules other than proteins for enzymic activity. The term holoenzyme refers to the active enzyme with its nonprotein component, whereas the enzyme without its nonprotein moiety is termed an apoenzyme and is inactive. If the nonprotein moiety is a metal ion, such as Zn^{2+} or Fe^{2+}, it is called a cofactor. If it is a small organic molecule, it is termed a coenzyme. Coenzymes that only transiently associate with the enzyme are called cosubstrates. Cosubstrates dissociate from the enzyme in an altered state (NAD^+ is an example, see p. 101). If the coenzyme is permanently associated with the enzyme and returned to its original form, it is called a prosthetic group (FAD is an example, see p. 110).

Coenzymes commonly are derived from vitamins. For example, NAD^+ contains niacin, and FAD contains riboflavin (see Chapter 28).

E. Regulation

Enzyme activity can be regulated, that is, increased or decreased, so that the rate of product formation responds to cellular need.

F. Location within the cell

Many enzymes are localized in specific organelles within the cell (Figure 5.3). Such compartmentalization serves to isolate the reaction substrate or product from other competing reactions. This provides a favorable environment for the reaction and organizes the thousands of enzymes present in the cell into purposeful pathways.

IV. HOW ENZYMES WORK

The mechanism of enzyme action can be viewed from two different perspectives. The first treats catalysis in terms of energy changes that occur during the reaction. That is, enzymes provide an alternate, energetically favorable reaction pathway different from the uncatalyzed reaction. The second perspective describes how the active site chemically facilitates catalysis.

A. Energy changes occurring during the reaction

Virtually all chemical reactions have an energy barrier separating the reactants and the products. This barrier, called the free energy of activation, is the energy difference between that of the reactants and a high-energy intermediate that occurs during the formation of product. For example, Figure 5.4 shows the changes in energy during the conversion of a molecule of reactant A to product B as it proceeds through the transition state (high-energy intermediate), T*:

$$A \rightleftarrows T^* \rightleftarrows B$$

1. **Free energy of activation:** The peak of energy in Figure 5.4 is the difference in free energy between the reactant and T*, where the high-energy intermediate is formed during the conversion of reactant to product. Because of the high free energy of activation, the rates of uncatalyzed chemical reactions are often slow.
2. **Rate of reaction:** For molecules to react, they must contain sufficient energy to overcome the energy barrier of the transition state. In the absence of an enzyme, only a small proportion of a population of molecules may possess enough energy to achieve the transition state between reactant and product. The rate of reaction is determined by the number of such energized molecules. In general, the lower the free energy of activation, the more molecules have sufficient energy to pass through the transition state, and, therefore, the faster the rate of the reaction.
3. **Alternate reaction pathway:** An enzyme allows a reaction to proceed rapidly under conditions prevailing in the cell by providing an alternate reaction pathway with a lower free energy of activation (see Figure 5.4). The enzyme does not change the free energies of the reactants or products and, therefore, does not change the equilibrium of the reaction (see p. 70). It does, however, accelerate the rate by which equilibrium is reached.

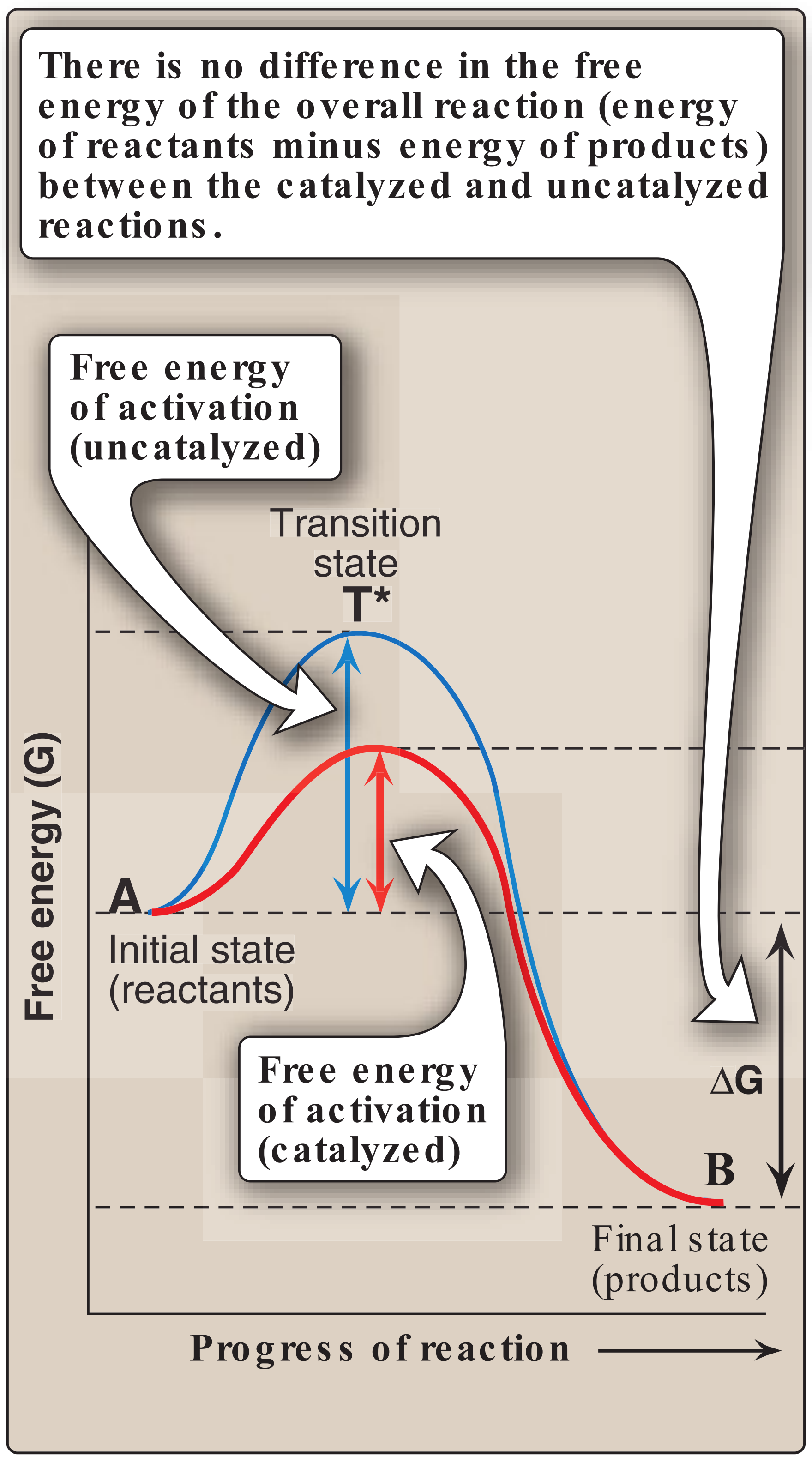

There is no difference in the free energy of the overall reaction (energy of reactants minus energy of products) between the catalyzed and uncatalyzed reactions.
Free energy of activation (uncatalyzed)
Transition state
T*
Free energy (G)
A
Initial state (reactants)
Free energy of activation (catalyzed)
ΔG
B
Final state (products)
Progress of reaction

Figure 5.4
Effect of an enzyme on the activation energy of a reaction.

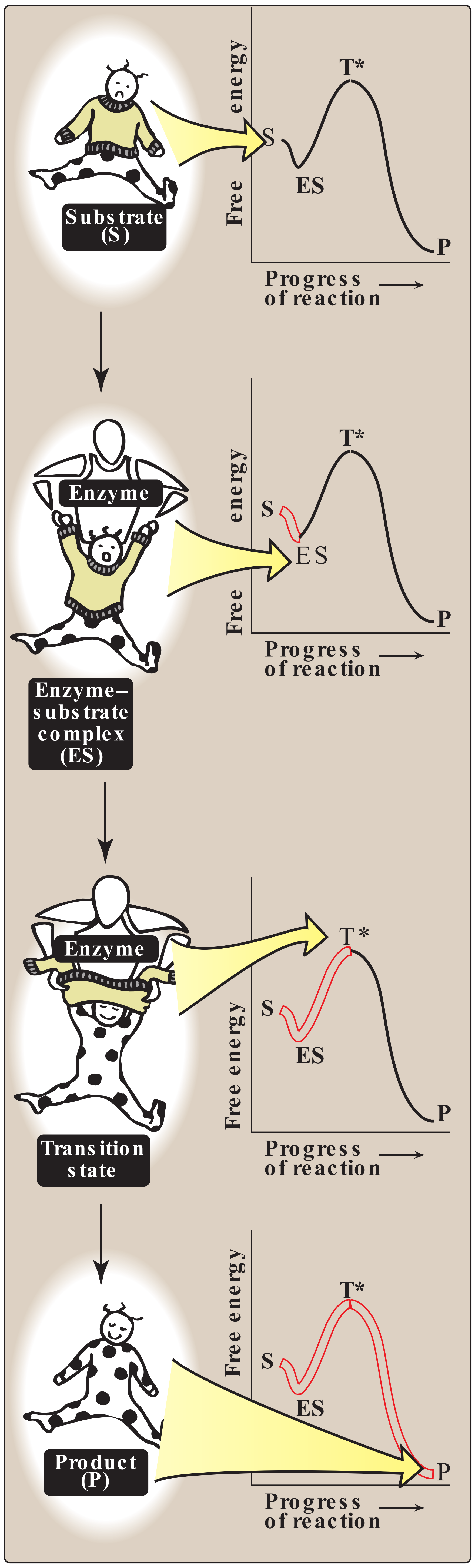


Figure 5.5
Schematic representation of energy changes accompanying formation of an enzyme–substrate complex and subsequent formation of a transition state.

B. Chemistry of the active site

The active site is not a passive receptacle for binding the substrate but, rather, is a complex molecular machine employing a diversity of chemical mechanisms to facilitate the conversion of substrate to product. A number of factors are responsible for the catalytic efficiency of enzymes, including the following examples.

1. **Transition-state stabilization:** The active site often acts as a flexible molecular template that binds the substrate and initiates its conversion to the transition state, a structure in which the bonds are not like those in the substrate or the product (see T* at the top of the curve in Figure 5.4). By stabilizing the transition state, the enzyme greatly increases the concentration of the reactive intermediate that can be converted to product and, thus, accelerates the reaction. [Note: The transition state cannot be isolated.]

2. **Other mechanisms:** The active site can provide catalytic groups that enhance the probability that the transition state is formed. In some enzymes, these groups can participate in general acid–base catalysis in which amino acid residues provide or accept protons. In other enzymes, catalysis may involve the transient formation of a covalent ES complex. [Note: The mechanism of action of chymotrypsin, an enzyme of protein digestion in the intestine, includes general base, general acid, and covalent catalysis. A histidine at the active site of the enzyme gains (general base) and loses (general acid) protons, mediated by the pK of histidine in proteins being close to physiologic pH. Serine at the active site forms a covalent link with the substrate.]

3. **Visualization of the transition state:** The enzyme-catalyzed conversion of substrate to product can be visualized as being similar to removing a sweater from an uncooperative infant (Figure 5.5). The process has a high energy of activation because the only reasonable strategy for removing the garment (short of ripping it off) requires that the random flailing of the baby results in both arms being fully extended over the head, an unlikely posture. However, we can envision a parent acting as an enzyme, first coming in contact with the baby (forming ES), then guiding the baby's arms into an extended, vertical position, analogous to the ES transition state. This posture (conformation) of the baby facilitates the removal of the sweater, forming the disrobed baby, which here represents product. [Note: The substrate bound to the enzyme (ES) is at a slightly lower energy than unbound substrate (S) and explains the small "dip" in the curve at ES.]

V. FACTORS AFFECTING REACTION VELOCITY

Enzymes can be isolated from cells and their properties studied in a test tube (that is, in vitro). Different enzymes show different responses to changes in substrate concentration, temperature, and pH. This section describes factors that influence the reaction velocity of enzymes. Enzymic responses to these factors give us valuable clues as to how enzymes function in living cells (that is, in vivo).

A. Substrate concentration

1. **Maximal velocity:** The rate or velocity of a reaction (v) is the number of substrate molecules converted to product per unit time. Velocity is usually expressed as µmol of product formed per minute. The rate of an enzyme-catalyzed reaction increases with substrate concentration until a maximal velocity (V_{max}) is reached (Figure 5.6). The leveling off of the reaction rate at high substrate concentrations reflects the saturation with substrate of all available binding sites on the enzyme molecules present.

2. **Hyperbolic shape of the enzyme kinetics curve:** Most enzymes show Michaelis-Menten kinetics (see p. 58), in which the plot of initial reaction velocity (v_o) against substrate concentration ([S]), is hyperbolic (similar in shape to that of the oxygen-dissociation curve of myoglobin, see p. 29). In contrast, allosteric enzymes do not follow Michaelis-Menton kinetics and show a sigmoidal curve (see p. 62) that is similar in shape to the oxygen-dissociation curve of hemoglobin (see p. 29).

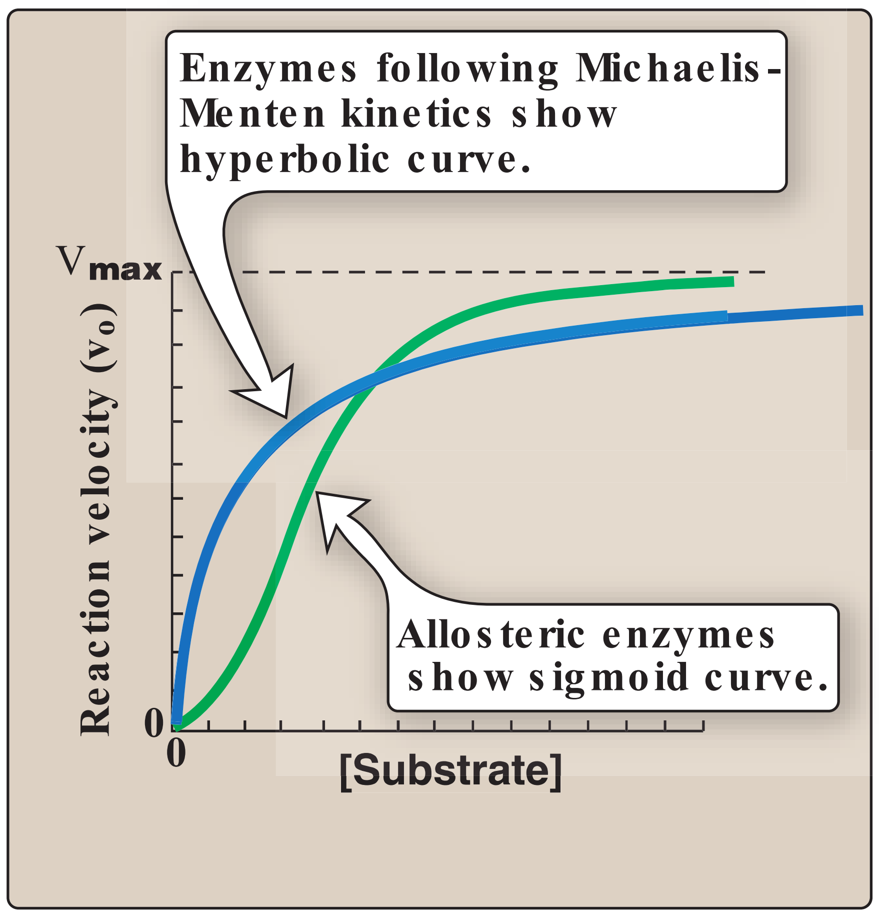


Figure 5.6
Effect of substrate concentration on reaction velocity.

B. Temperature

1. **Increase of velocity with temperature:** The reaction velocity increases with temperature until a peak velocity is reached (Figure 5.7). This increase is the result of the increased number of molecules having sufficient energy to pass over the energy barrier and form the products of the reaction.

2. **Decrease of velocity with higher temperature:** Further elevation of the temperature causes a decrease in reaction velocity as a result of temperature-induced denaturation of the enzyme (see Figure 5.7).

> The optimum temperature for most human enzymes is between 35°C and 40°C. Human enzymes start to denature at temperatures above 40°C, but thermophilic bacteria found in the hot springs have optimum temperatures of 70°C.

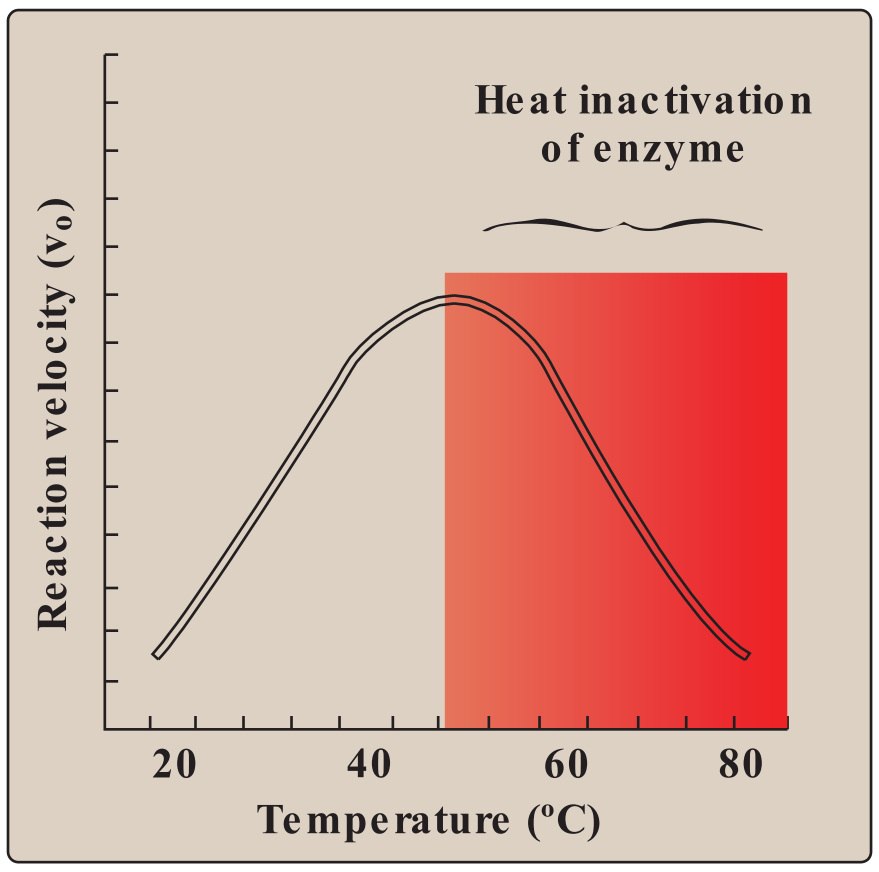


Figure 5.7
Effect of temperature on an enzyme-catalyzed reaction.

C. pH

1. **Effect of pH on the ionization of the active site:** The concentration of protons (H^+) affects reaction velocity in several ways. First, the catalytic process usually requires that the enzyme and substrate have specific chemical groups in either an ionized or un-ionized state in order to interact. For example, catalytic activity may require that an amino group of the enzyme be in the protonated form ($-NH_3^+$). At alkaline pH, this group is deprotonated, and the rate of the reaction, therefore, declines.

2. **Effect of pH on enzyme denaturation:** Extremes of pH can also lead to denaturation of the enzyme, because the structure of the catalytically active protein molecule depends on the ionic character of the amino acid side chains.

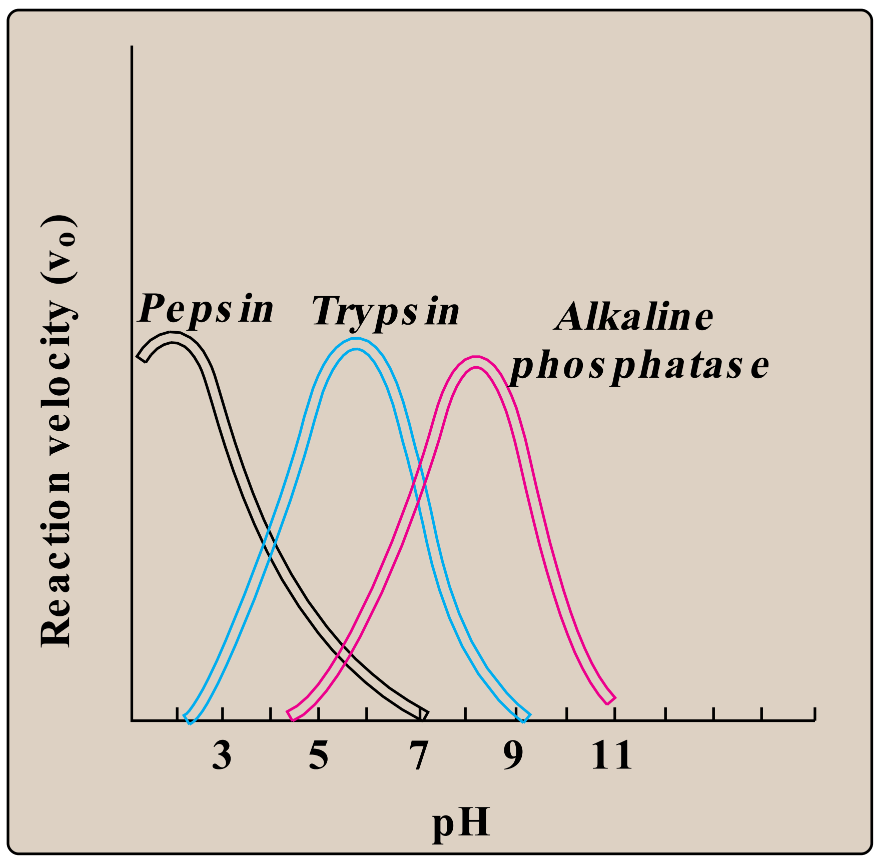


Figure 5.8
Effect of pH on enzyme-catalyzed reactions.

3. **Variable pH optimum:** The pH at which maximal enzyme activity is achieved is different for different enzymes and often reflects the [H^+] at which the enzyme functions in the body. For example, pepsin, a digestive enzyme in the stomach, is maximally active at pH 2, whereas other enzymes, designed to work at neutral pH, are denatured by such an acidic environment (Figure 5.8).

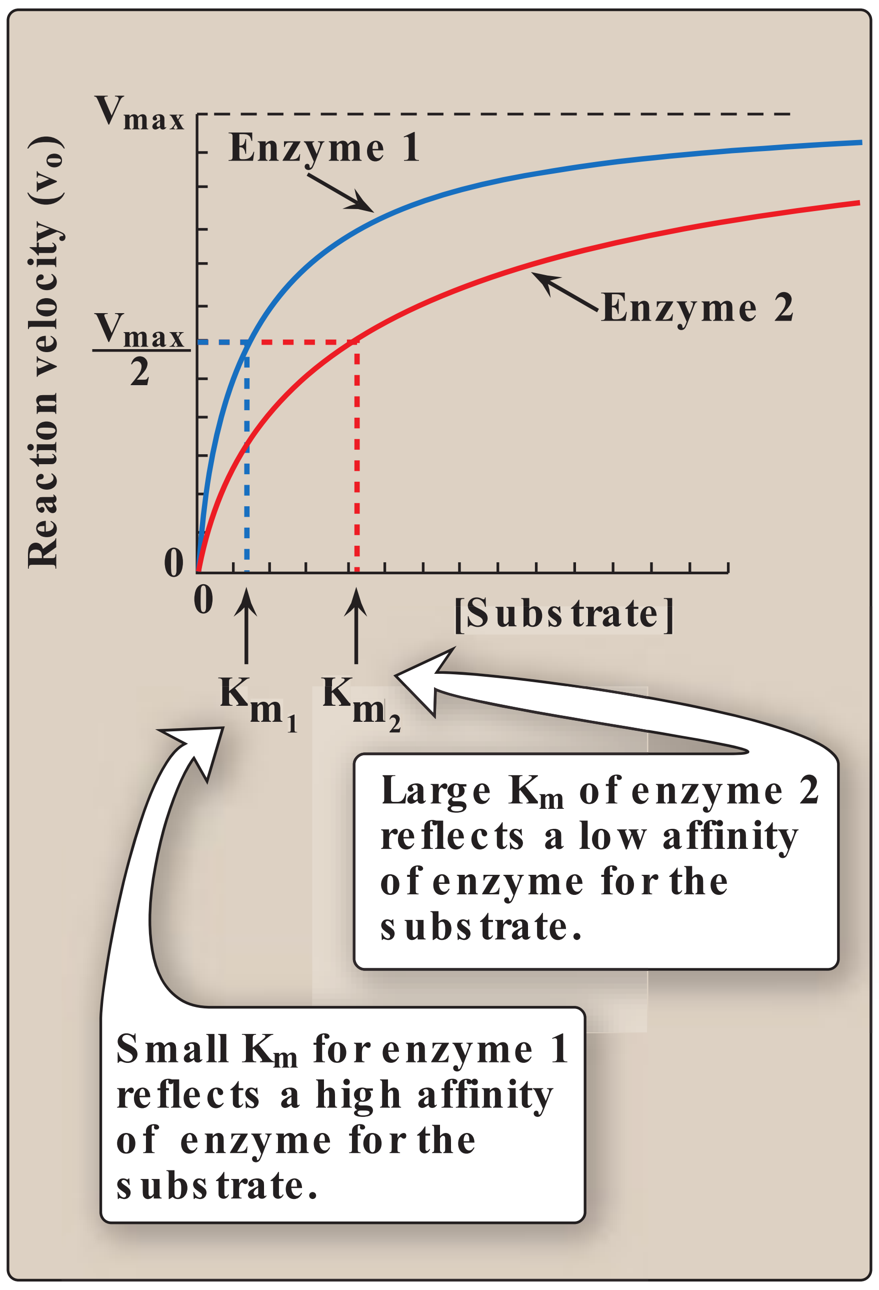
VI. MICHAELIS-MENTEN KINETICS

A. Reaction model

Leonor Michaelis and Maude Menten proposed a simple model that accounts for most of the features of enzyme-catalyzed reactions. In this model, the enzyme reversibly combines with its substrate to form an ES complex that subsequently yields product, regenerating the free enzyme. The model, involving one substrate molecule, is represented below:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

where S is the substrate
E is the enzyme
ES is the enzyme–substrate complex
P is the product
k_1, k_{-1}, and k_2 are rate constants



Large K_m of enzyme 2 reflects a low affinity of enzyme for the substrate.

Small K_m for enzyme 1 reflects a high affinity of enzyme for the substrate.

Figure 5.9
Effect of substrate concentration on reaction velocities for two enzymes: enzyme 1 with a small Michaelis constant (K_m) and enzyme 2 with a large K_m. V_{max} = maximal velocity.

B. Michaelis-Menten equation

The Michaelis-Menten equation describes how reaction velocity varies with substrate concentration:

$$v_o = \frac{V_{max}\,[S]}{K_m + [S]}$$

where V_o = initial reaction velocity
V_{max} = maximal velocity
K_m = Michaelis constant = $(k_{-1} + k_2)/k_1$
[S] = substrate concentration

The following assumptions are made in deriving the Michaelis-Menten rate equation:

1. **Relative concentrations of enzyme and substrate:** The concentration of substrate ([S]) is much greater than the concentration of enzyme ([E]), so that the percentage of total substrate bound by the enzyme at any one time is small.

2. **Steady-state assumption:** [ES] does not change with time (the steady-state assumption), that is, the rate of formation of ES is equal to that of the breakdown of ES (to E + S and to E + P). In general, an intermediate in a series of reactions is said to be in steady state when its rate of synthesis is equal to its rate of degradation.

3. **Initial velocity:** Initial reaction velocities (v_o) are used in the analysis of enzyme reactions. This means that the rate of the reaction is measured as soon as enzyme and substrate are mixed. At that time, the concentration of product is very small, and, therefore, the rate of the back reaction from product to substrate can be ignored.

C. Important conclusions

1. **Characteristics of K_m:** K_m, the Michaelis constant, is characteristic of an enzyme and its particular substrate and reflects the affinity of the enzyme for that substrate. K_m is numerically equal to the substrate concentration at which the reaction velocity is equal to $\frac{1}{2}V_{max}$. K_m does not vary with enzyme concentration.

    a. **Small K_m:** A numerically small (low) K_m reflects a high affinity of the enzyme for substrate, because a low concentration of substrate is needed to half-saturate the enzyme—that is, to reach a velocity that is $\frac{1}{2}V_{max}$ (Figure 5.9).

    b. **Large K_m:** A numerically large (high) K_m reflects a low affinity of enzyme for substrate because a high concentration of substrate is needed to half-saturate the enzyme.

2. **Relationship of velocity to enzyme concentration:** The rate of the reaction is directly proportional to the enzyme concentration at all substrate concentrations. For example, if the enzyme concentration is halved, the initial rate of the reaction (v_o), as well as that of V_{max}, are reduced to half that of the original.

3. **Order of reaction:** When [S] is much less than K_m, the velocity of the reaction is approximately proportional to the substrate concentration (Figure 5.10). The rate of reaction is then said to be first order with respect to substrate. When [S] is much greater than K_m, the velocity is constant and equal to V_{max}. The rate of reaction is then independent of substrate concentration (the enzyme is saturated with substrate) and is said to be zero order with respect to substrate concentration (see Figure 5.10).

D. Lineweaver-Burk plot

When v_o is plotted against [S], it is not always possible to determine when V_{max} has been achieved because of the gradual upward slope of the hyperbolic curve at high substrate concentrations. However, if $1/v_o$ is plotted versus $1/[S]$, a straight line is obtained (Figure 5.11). This plot, the Lineweaver-Burk plot (also called a double-reciprocal plot) can be used to calculate K_m and V_{max} as well as to determine the mechanism of action of enzyme inhibitors.

1. The equation describing the Lineweaver-Burk plot is:

$$\frac{1}{v_o} = \frac{K_m}{V_{max}\,[S]} + \frac{1}{V_{max}}$$

VII. INHIBITION OF ENZYME ACTIVITY

Any substance that can decrease the velocity of an enzyme-catalyzed reaction is called an inhibitor. Inhibitors can be reversible or irreversible. Irreversible inhibitors bind to enzymes through covalent bonds. Lead, for example, forms covalent bonds with the sulfhydryl side chain of cysteine in proteins. Ferrochelatase, an enzyme involved in heme synthesis (see p. 279), is irreversibly inhibited by lead. [Note: An important group of irreversible inhibitors are the mechanism-based inhibitors that are converted by the enzyme itself to a form that covalently links to the enzyme, thereby inhibiting it. They also are referred to as “suicide” inhibitors.] Reversible inhibitors bind to enzymes through noncovalent bonds and, thus, dilution of the enzyme–inhibitor complex results in dissociation of the reversibly bound inhibitor and recovery of enzyme activity. The two most commonly encountered types of reversible inhibition are competitive and noncompetitive.

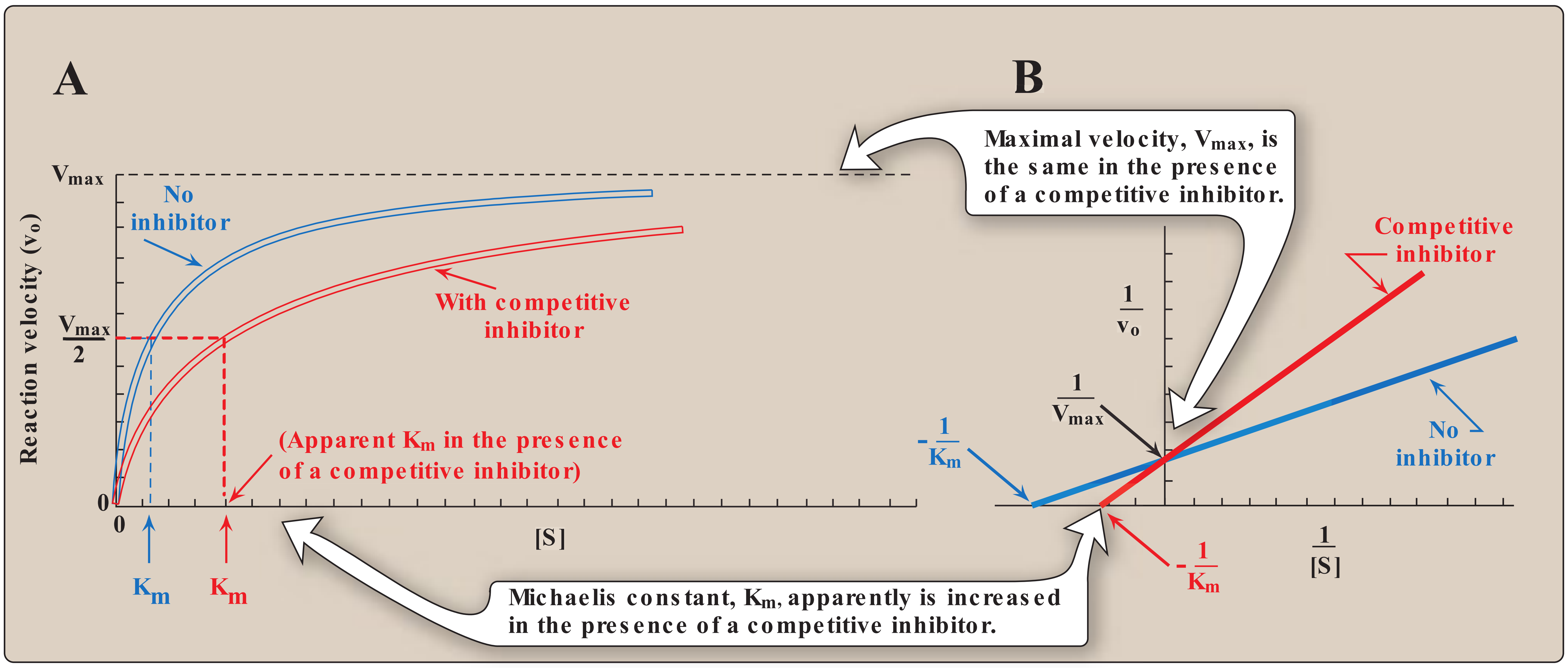
A. Competitive inhibition

This type of inhibition occurs when the inhibitor binds reversibly to the same site that the substrate would normally occupy and, therefore, competes with the substrate for that site.

1. **Effect on V_{max}:** The effect of a competitive inhibitor is reversed by increasing [S]. At a sufficiently high substrate concentration, the reaction velocity reaches the V_{max} observed in the absence of inhibitor (Figure 5.12).

2. **Effect on K_m:** A competitive inhibitor increases the apparent K_m for a given substrate. This means that, in the presence of a competitive inhibitor, more substrate is needed to achieve $\frac{1}{2}V_{max}$.

3. **Effect on the Lineweaver-Burk plot:** Competitive inhibition shows a characteristic Lineweaver-Burk plot in which the plots of the inhibited



Maximal velocity, V_{max}, is the same in the presence of a competitive inhibitor.

Michaelis constant, K_m, apparently is increased in the presence of a competitive inhibitor.

Figure 5.12
A. Effect of a competitive inhibitor on the reaction velocity versus substrate ([S]) plot. B. Lineweaver-Burk plot of competitive inhibition of an enzyme.

and uninhibited reactions intersect on the y axis at $1/V_{max}$ (V_{max} is unchanged). The inhibited and uninhibited reactions show different x-axis intercepts, indicating that the apparent K_m is increased in the presence of the competitive inhibitor because $-1/K_m$ moves closer to zero from a negative value (see Figure 5.12). [Note: An important group of competitive inhibitors are the transition state analogs, stable molecules that approximate the structure of the transition state and, therefore, bind the enzyme with a higher affinity than the substrate.]

4. **Statin drugs as examples of competitive inhibitors:** This group of antihyperlipidemic agents competitively inhibits the rate-limiting (slowest) step in cholesterol biosynthesis. This reaction is catalyzed by hydroxymethylglutaryl–CoA reductase (HMG-CoA reductase, see p. 220). Statins, such as atorvastatin (Lipitor) and pravastatin (Pravachol), are structural analogs of the natural substrate for this enzyme and compete effectively to inhibit HMG-CoA reductase. By doing so, they inhibit de novo cholesterol synthesis, thereby lowering plasma cholesterol levels (Figure 5.13).

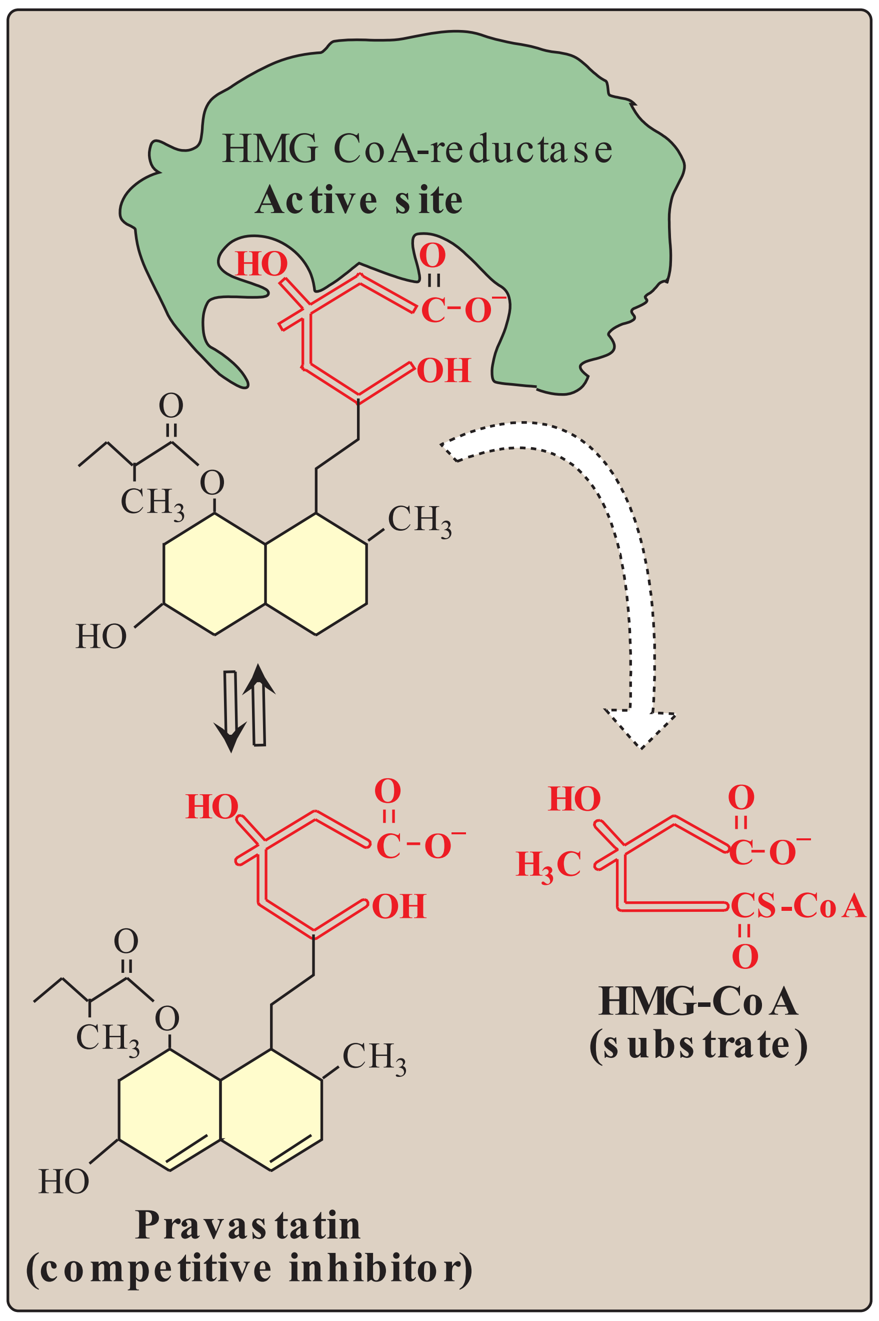


Figure 5.13
Pravastatin competes with HMG-CoA for the active site of HMG-CoA reductase. HMG-CoA = hydroxymethylglutaryl–coenzyme A.

B. Noncompetitive inhibition

This type of inhibition is recognized by its characteristic effect on V_{max} (Figure 5.14). Noncompetitive inhibition occurs when the inhibitor and substrate bind at different sites on the enzyme. The noncompetitive inhibitor can bind either free enzyme or the enzyme-substrate complex, thereby preventing the reaction from occurring (Figure 5.15).

1. **Effect on V_{max}:** Noncompetitive inhibition cannot be overcome by increasing the concentration of substrate. Therefore, noncompetitive inhibitors decrease the apparent V_{max} of the reaction.

2. **Effect on K_m:** Noncompetitive inhibitors do not interfere with the binding of substrate to enzyme. Therefore, the enzyme shows the same K_m in the presence or absence of the noncompetitive inhibitor.

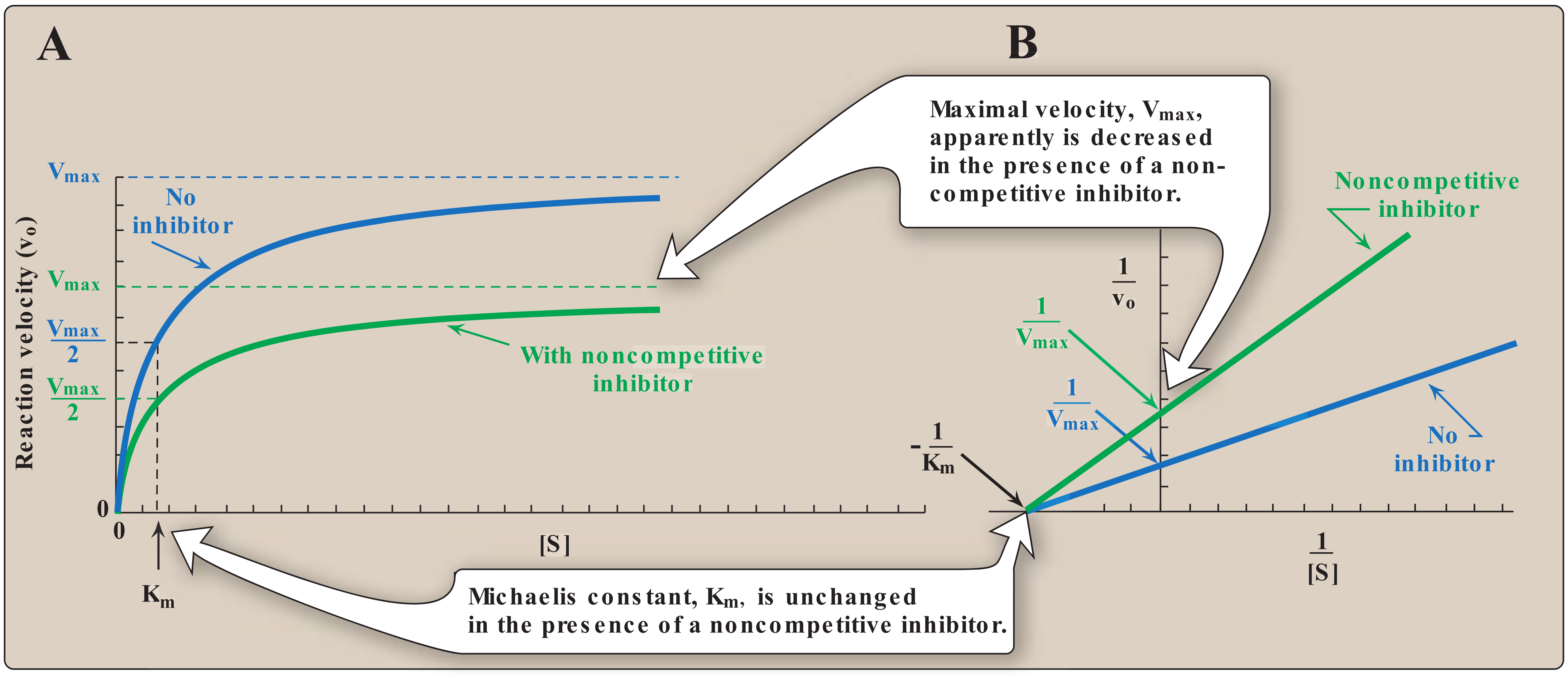


Figure 5.14
A. Effect of a noncompetitive inhibitor on the reaction velocity versus substrate ([S]) plot. B. Lineweaver-Burk plot of noncompetitive inhibition of an enzyme.

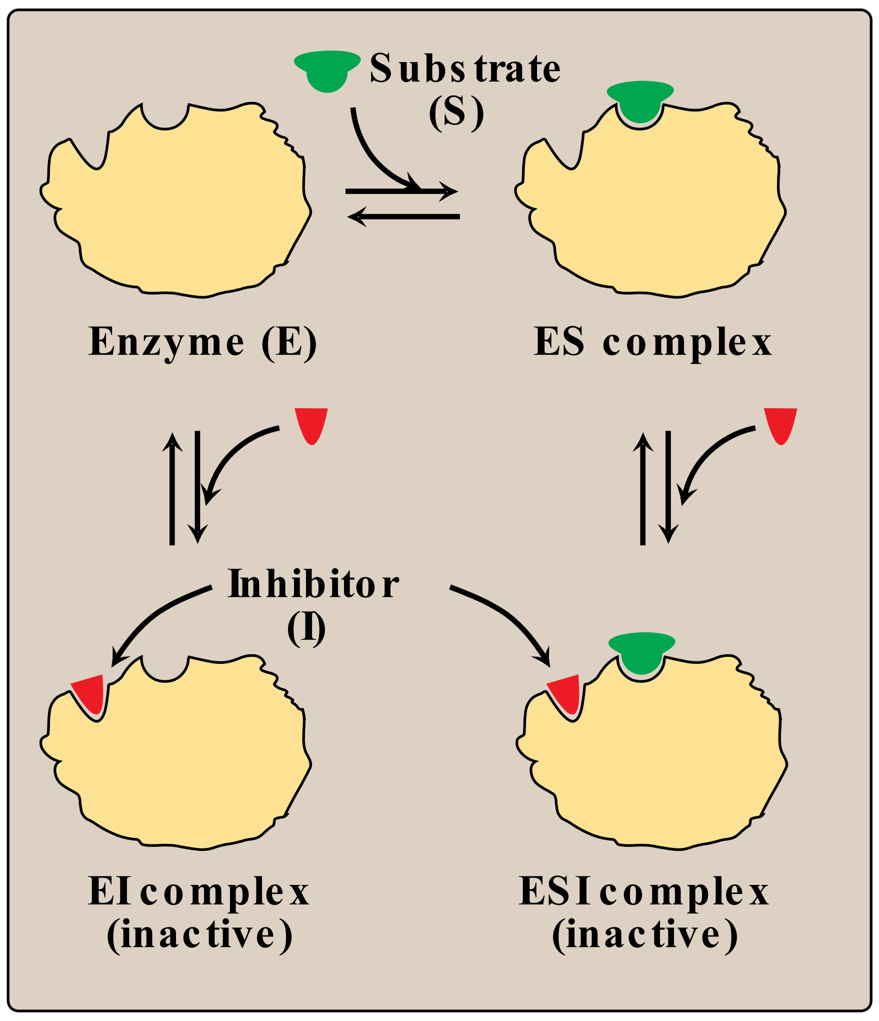


Figure 5.15
A noncompetitive inhibitor binding to both free enzyme and enzyme–substrate (ES) complex.

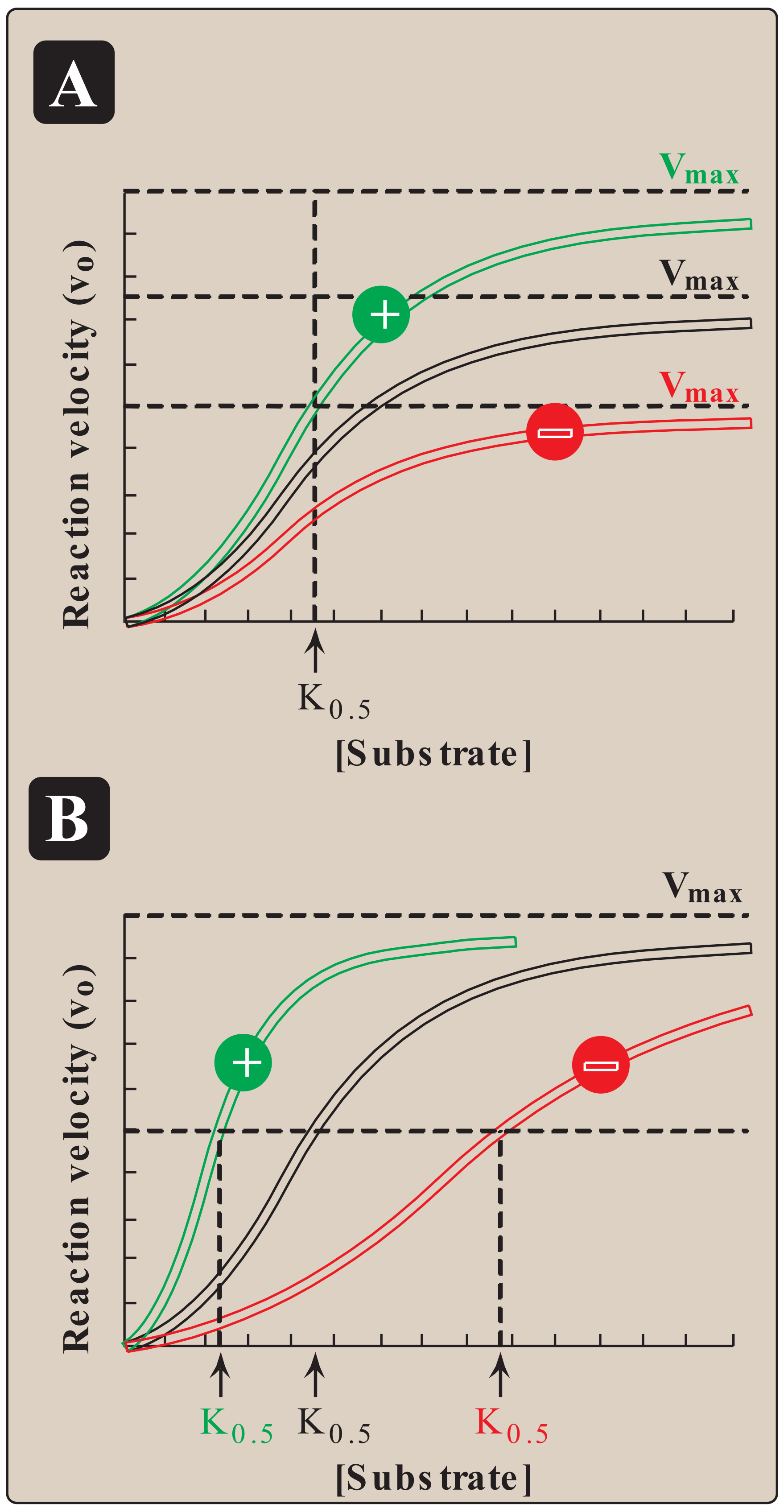


Figure 5.16
Effects of negative ⊖ or positive ⊕ effectors on an allosteric enzyme. A. V_{max} is altered. B. The substrate concentration that gives half-maximal velocity ($K_{0.5}$) is altered.

3. **Effect on Lineweaver-Burk plot:** Noncompetitive inhibition is readily differentiated from competitive inhibition by plotting $1/v_o$ versus 1/[S] and noting that the apparent V_{max} decreases in the presence of a noncompetitive inhibitor, whereas K_m is unchanged (see Figure 5.14). [Note: Oxypurinol, a metabolite of the drug allopurinol, is a noncompetitive inhibitor of xanthine oxidase, an enzyme of purine degradation (see p. 301).]

C. Enzyme inhibitors as drugs

At least half of the ten most commonly prescribed drugs in the United States act as enzyme inhibitors. For example, the widely prescribed β-lactam antibiotics, such as penicillin and amoxicillin, act by inhibiting enzymes involved in bacterial cell wall synthesis. Drugs may also act by inhibiting extracellular reactions. This is illustrated by angiotensin-converting enzyme (ACE) inhibitors. They lower blood pressure by blocking the enzyme that cleaves angiotensin I to form the potent vasoconstrictor, angiotensin II. These drugs, which include captopril, enalapril, and lisinopril, cause vasodilation and, therefore, a reduction in blood pressure. Aspirin, a nonprescription drug, irreversibly inhibits prostaglandin and thromboxane synthesis (see p. 214).

VIII. REGULATION OF ENZYME ACTIVITY

The regulation of the reaction velocity of enzymes is essential if an organism is to coordinate its numerous metabolic processes. The rates of most enzymes are responsive to changes in substrate concentration, because the intracellular level of many substrates is in the range of the K_m. Thus, an increase in substrate concentration prompts an increase in reaction rate, which tends to return the concentration of substrate toward normal. In addition, some enzymes with specialized regulatory functions respond to allosteric effectors and/or covalent modification or they show altered rates of enzyme synthesis (or degradation) when physiologic conditions are changed.

A. Regulation of allosteric enzymes

Allosteric enzymes are regulated by molecules called effectors that bind noncovalently at a site other than the active site. These enzymes are almost always composed of multiple subunits, and the regulatory (allosteric) site that binds the effector is distinct from the substrate-binding site and may be located on a subunit that is not itself catalytic. Effectors that inhibit enzyme activity are termed negative effectors, whereas those that increase enzyme activity are called positive effectors. Positive and negative effectors can affect the affinity of the enzyme for its substrate ($K_{0.5}$), modify the maximal catalytic activity of the enzyme (V_{max}), or both (Figure 5.16). [Note: Allosteric enzymes frequently catalyze the committed step early in a pathway.]

1. **Homotropic effectors:** When the substrate itself serves as an effector, the effect is said to be homotropic. Most often, an allosteric substrate functions as a positive effector. In such a case, the pres-

ence of a substrate molecule at one site on the enzyme enhances the catalytic properties of the other substrate-binding sites. That is, their binding sites exhibit cooperativity. These enzymes show a sigmoidal curve when reaction velocity (v_o) is plotted against substrate concentration ([S]), as shown in Figure 5.16. This contrasts with the hyperbolic curve characteristic of enzymes following Michaelis-Menten kinetics, as previously discussed. [Note: The concept of cooperativity of substrate binding is analogous to the binding of oxygen to hemoglobin (see p. 29).]

2. **Heterotropic effectors:** The effector may be different from the substrate, in which case the effect is said to be heterotropic. For example, consider the feedback inhibition shown in Figure 5.17. The enzyme that converts D to E has an allosteric site that binds the endproduct, G. If the concentration of G increases (for example, because it is not used as rapidly as it is synthesized), the first irreversible step unique to the pathway is typically inhibited. Feedback inhibition provides the cell with appropriate amounts of a product it needs by regulating the flow of substrate molecules through the pathway that synthesizes that product. Heterotropic effectors are commonly encountered. For example, the glycolytic enzyme phosphofructokinase-1 is allosterically inhibited by citrate, which is not a substrate for the enzyme (see p. 99).

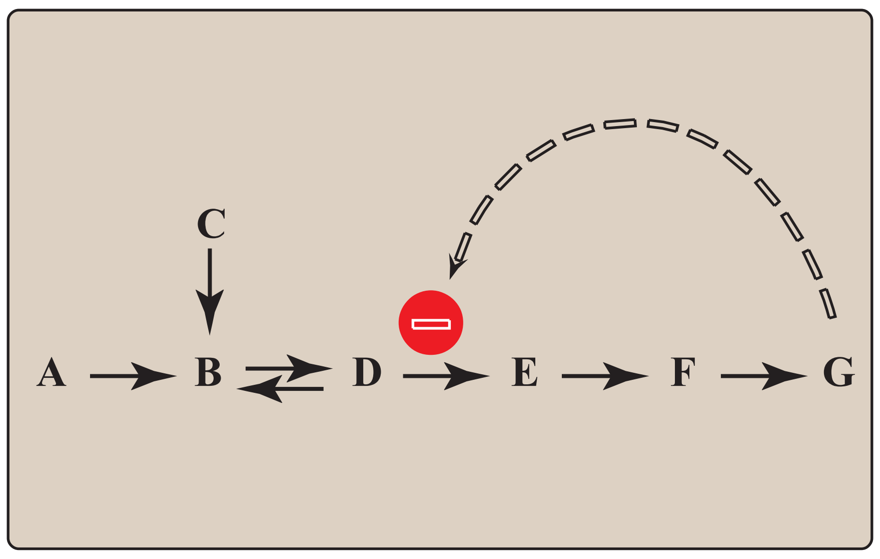


Figure 5.17
Feedback inhibition of a metabolic pathway.

B. Regulation of enzymes by covalent modification

Many enzymes are regulated by covalent modification, most often by the addition or removal of phosphate groups from specific serine, threonine, or tyrosine residues of the enzyme. Protein phosphorylation is recognized as one of the primary ways in which cellular processes are regulated. [Note: Protein phosphorylation is mediated by hormonal signals (see p. 132).]

1. **Phosphorylation and dephosphorylation:** Phosphorylation reactions are catalyzed by a family of enzymes called protein kinases that use ATP as the phosphate donor. Phosphate groups are cleaved from phosphorylated enzymes by the action of phosphoprotein phosphatases (Figure 5.18).

2. **Response of enzyme to phosphorylation:** Depending on the specific enzyme, the phosphorylated form may be more or less active than the unphosphorylated enzyme. For example, phosphorylation of glycogen phosphorylase (an enzyme that degrades glycogen) increases activity, whereas phosphorylation of glycogen synthase (an enzyme that synthesizes glycogen) decreases activity (p. 132).

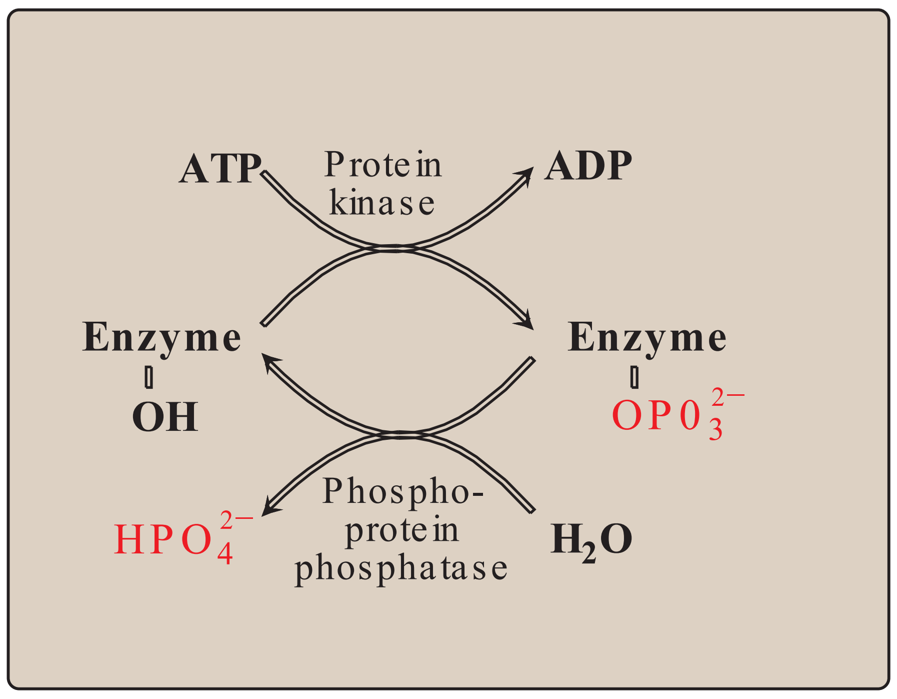


Figure 5.18
Covalent modification by the addition and removal of phosphate groups. [Note: HPO_4^{2-} may be represented as P_i.]

C. Induction and repression of enzyme synthesis

The regulatory mechanisms described above modify the activity of existing enzyme molecules. However, cells can also regulate the amount of enzyme present by altering the rate of enzyme degradation or, more typically, the rate of enzyme synthesis. The increase (induction) or decrease (repression) of enzyme synthesis leads to an alteration in the total population of active sites. Enzymes subject to

REGULATOR EVENT	TYPICAL EFFECTOR	RESULTS	TIME REQUIRED FOR CHANGE
Substrate availability	**Substrate**	**Change in velocity (v_o)**	**Immediate**
Product inhibition	**Reaction product**	**Change in V_{max} and/or K_m**	**Immediate**
Allosteric control	**Pathway end product**	**Change in V_{max} and/or $K_{0.5}$**	**Immediate**
Covalent modification	**Another enzyme**	**Change in V_{max} and/or K_m**	**Immediate to minutes**
Synthesis or degradation of enzyme	**Hormone or metabolite**	**Change in the amount of enzyme**	**Hours to days**

Figure 5.19
Mechanisms for regulating enzyme activity. [Note: Inhibition by pathway end product is also referred to as feedback inhibition.]

regulation of synthesis are often those that are needed at only one stage of development or under selected physiologic conditions. For example, elevated levels of insulin as a result of high blood glucose levels cause an increase in the synthesis of key enzymes involved in glucose metabolism (see p. 105). In contrast, enzymes that are in constant use are usually not regulated by altering the rate of enzyme synthesis. Alterations in enzyme levels as a result of induction or repression of protein synthesis are slow (hours to days), compared with allosterically or covalently regulated changes in enzyme activity, which occur in seconds to minutes. Figure 5.19 summarizes the common ways that enzyme activity is regulated.

IX. ENZYMES IN CLINICAL DIAGNOSIS

Plasma enzymes can be classified into two major groups. First, a relatively small group of enzymes are actively secreted into the blood by certain cell types. For example, the liver secretes zymogens (inactive precursors) of the enzymes involved in blood coagulation. Second, a large number of enzyme species are released from cells during normal cell turnover. These enzymes almost always function intracellularly and have no physiologic use in the plasma. In healthy individuals, the levels of these enzymes are fairly constant and represent a steady state in which the rate of release from damaged cells into the plasma is balanced by an equal rate of removal from the plasma. Increased plasma levels of these enzymes may indicate tissue damage (Figure 5.20).

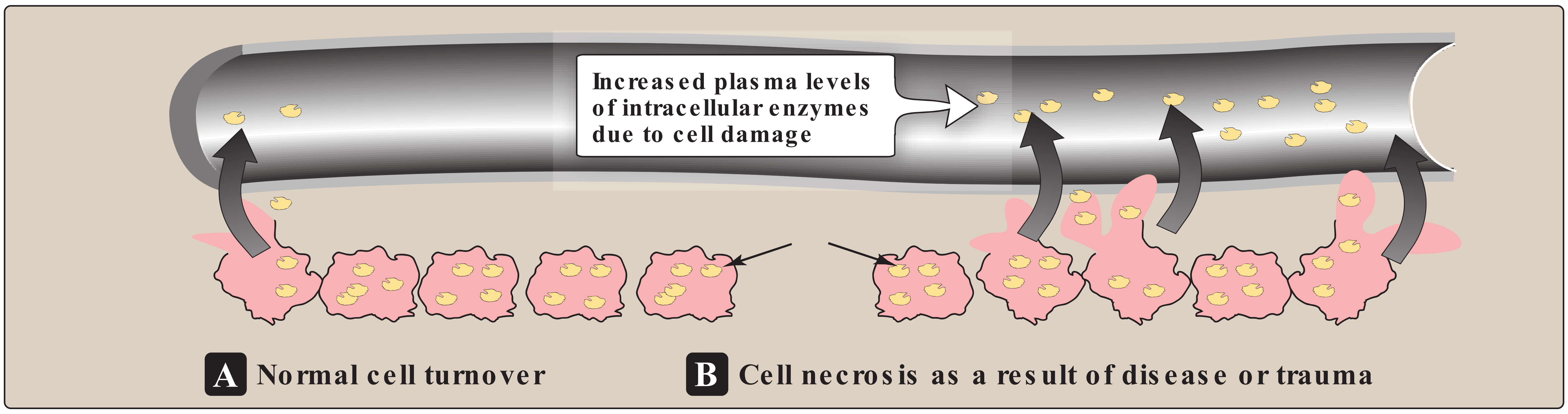

Increased plasma levels of intracellular enzymes due to cell damage
A Normal cell turnover
B Cell necrosis as a result of disease or trauma

Figure 5.20
Release of enzymes from normal and diseased or traumatized cells.

Plasma is the fluid, noncellular part of blood. Laboratory assays of enzyme activity most often use serum, which is obtained by centrifugation of whole blood after it has been allowed to coagulate. Plasma is a physiologic fluid, whereas serum is prepared in the laboratory.

A. Alteration of plasma enzyme levels in disease states

Many diseases that cause tissue damage result in an increased release of intracellular enzymes into the plasma. The activities of many of these enzymes are routinely determined for diagnostic purposes in diseases of the heart, liver, skeletal muscle, and other tissues. The level of specific enzyme activity in the plasma frequently correlates with the extent of tissue damage. Therefore, determining the degree of elevation of a particular enzyme activity in the plasma is often useful in evaluating the prognosis for the patient.

B. Plasma enzymes as diagnostic tools

Some enzymes show relatively high activity in only one or a few tissues. The presence of increased levels of these enzymes in plasma thus reflects damage to the corresponding tissue. For example, the enzyme alanine aminotransferase ([ALT] see p. 251) is abundant in the liver. The appearance of elevated levels of ALT in plasma signals possible damage to hepatic tissue. [Note: Measurement of ALT is part of the liver function test panel.] Increases in plasma levels of enzymes with a wide tissue distribution provide a less specific indication of the site of cellular injury and limits their diagnostic value.

C. Isoenzymes and diseases of the heart

Isoenzymes (also called isozymes) are enzymes that catalyze the same reaction. However, they do not necessarily have the same physical properties because of genetically determined differences in amino acid sequence. For this reason, isoenzymes may contain different numbers of charged amino acids and may, therefore, be separated from each other by electrophoresis (Figure 5.21). Different organs commonly contain characteristic proportions of different isoenzymes. The pattern of isoenzymes found in the plasma may, therefore, serve as a means of identifying the site of tissue damage. For example, the plasma levels of creatine kinase (CK) are commonly determined in the diagnosis of myocardial infarction. They are particularly useful when the electrocardiogram is difficult to interpret such as when there have been previous episodes of heart disease.

1. **Quaternary structure of isoenzymes:** Many isoenzymes contain different subunits in various combinations. For example, CK occurs as three isoenzymes. Each isoenzyme is a dimer composed of two polypeptides (called B and M subunits) associated in one of three combinations: CK1 = BB, CK2 = MB, and CK3 = MM. Each CK isoenzyme shows a characteristic electrophoretic mobility (see Figure 5.21). [Note: Virtually all CK in the brain is the BB isoform, whereas in skeletal muscle it is MM. In cardiac muscle, about one third is MB with the rest as MM.]

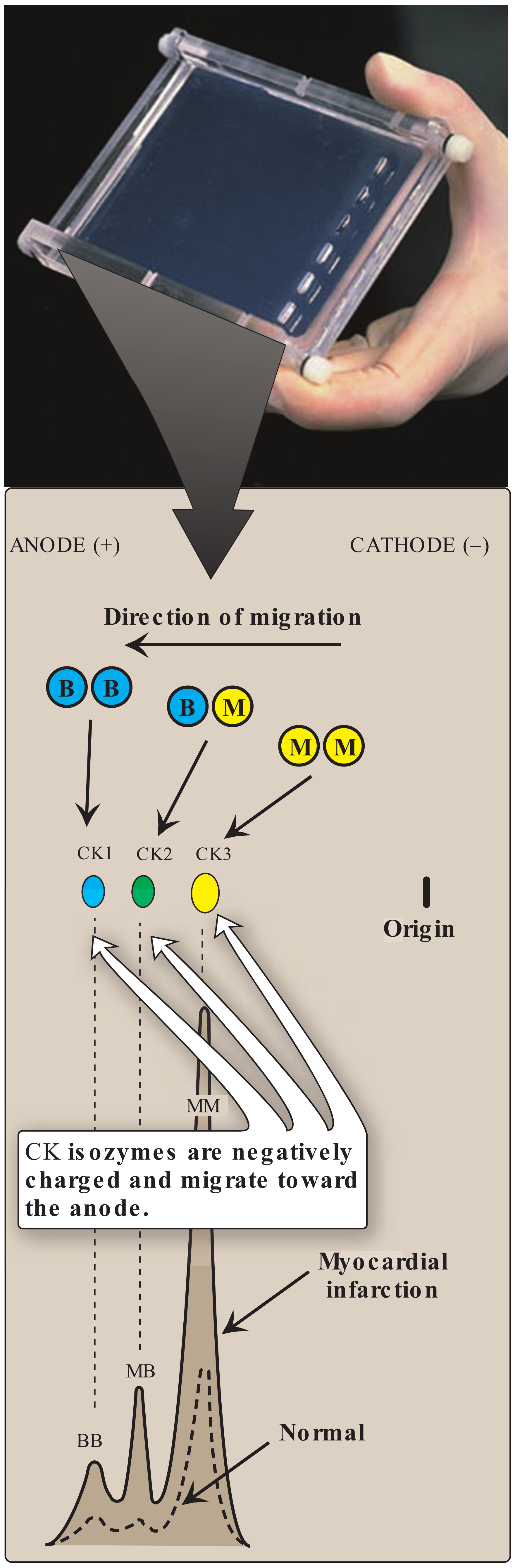


Figure 5.21
Subunit composition, electrophoretic mobility, and enzyme activity of creatine kinase (CK) isoenzymes.

2. **Diagnosis of myocardial infarction:** Measurement of blood levels of proteins with cardiac specificity (biomarkers) is used in the diagnosis of myocardial infarction (MI). Myocardial muscle is the only tissue that contains more than 5% of the total CK activity as the CK2 (MB) isoenzyme. Appearance of this hybrid isoenzyme in plasma is virtually specific for infarction of the myocardium. Following an acute MI, CK2 appears approximately 4–8 hours following onset of chest pain, reaches a peak of activity at approximately 24 hours, and returns to baseline after 48–72 hours (Figure 5.22). Troponin T and troponin I are regulatory proteins involved in myocardial contractility. They, too, are released into the plasma in response to cardiac damage. Cardiac troponin I (cTnI) is highly sensitive and specific for damage to cardiac tissue. cTnI appears in plasma within 4–6 hours after an MI, peaks in 8–28 hours, and remains elevated for 3–10 days. Elevated cTns, in combination with the clinical presentation and characteristic changes in the electrocardiogram, are currently considered the "gold standard" in the diagnosis of a MI.

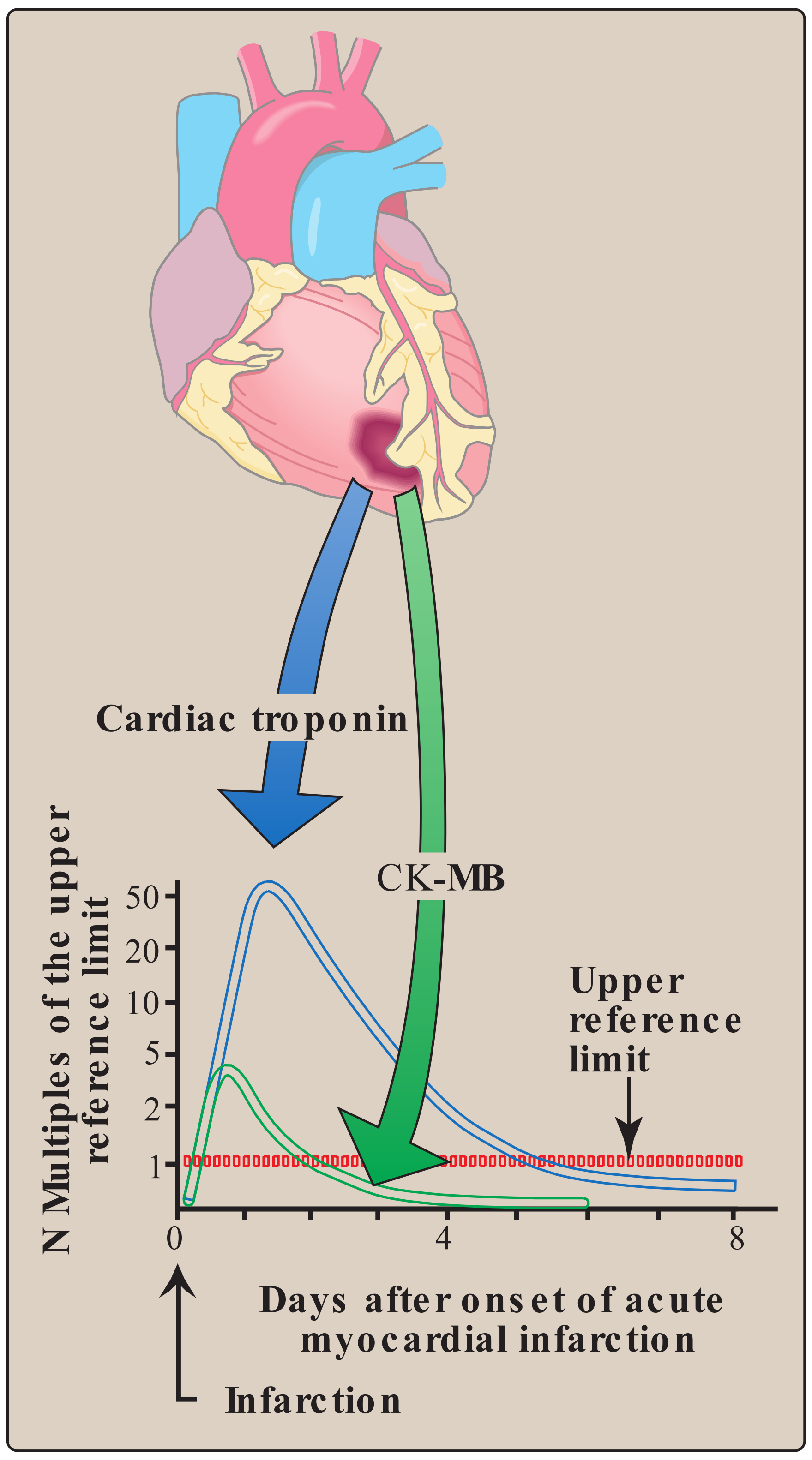


Figure 5.22
Appearance of creatine kinase isozyme CK-MB and cardiac troponin in plasma after a myocardial infarction.

X. CHAPTER SUMMARY

Enzymes are **protein catalysts** that increase the velocity of a chemical reaction by lowering the energy of the transition state (Figure 5.23). Enzymes are not consumed during the reaction they catalyze. Enzyme molecules contain a special pocket or cleft called the **active site**. The active site contains amino acid side chains that participate in substrate binding and catalysis. The active site binds the substrate, forming an **enzyme–substrate (ES) complex**. Binding is thought to cause a conformational change in the enzyme (induced fit) that allows catalysis. ES is converted to enzyme–product (EP), which subsequently dissociates to enzyme and product. An enzyme allows a reaction to proceed rapidly under conditions prevailing in the cell by providing an **alternate reaction pathway** with a **lower free energy of activation**. The enzyme does not change the free energies of the reactants or products and, therefore, does not change the equilibrium of the reaction. Most enzymes show **Michaelis-Menten kinetics**, and a plot of the **initial reaction velocity** (v_o) against **substrate concentration** (**[S]**) has a **hyperbolic** shape similar to the oxygen-dissociation curve of myoglobin. Any substance that can diminish the velocity of such enzyme-catalyzed reactions is called an inhibitor. The two most commonly encountered types of reversible inhibition are **competitive** (which **increases** the **apparent K_m**) and **noncompetitive** (which **decreases** the **apparent V_{max}**). In contrast, the **multisubunit allosteric enzymes** frequently show a **sigmoidal curve** similar in shape to the oxygen-dissociation curve of hemoglobin. They typically catalyze the **rate-limiting** (slowest step) of a pathway. Allosteric enzymes are regulated by molecules called **effectors** that bind noncovalently at a site other than the active site. Effectors can be either **positive** (accelerate the enzyme-catalyzed reaction) or **negative** (slow down the reaction). An allosteric effector can alter the affinity of the enzyme for its substrate, modify the maximal catalytic activity of the enzyme, or both. Enzymes can also be regulated by covalent modification and by changes in the rate of synthesis or degradation. Enzymes have diagnostic and therapeutic value in medicine.

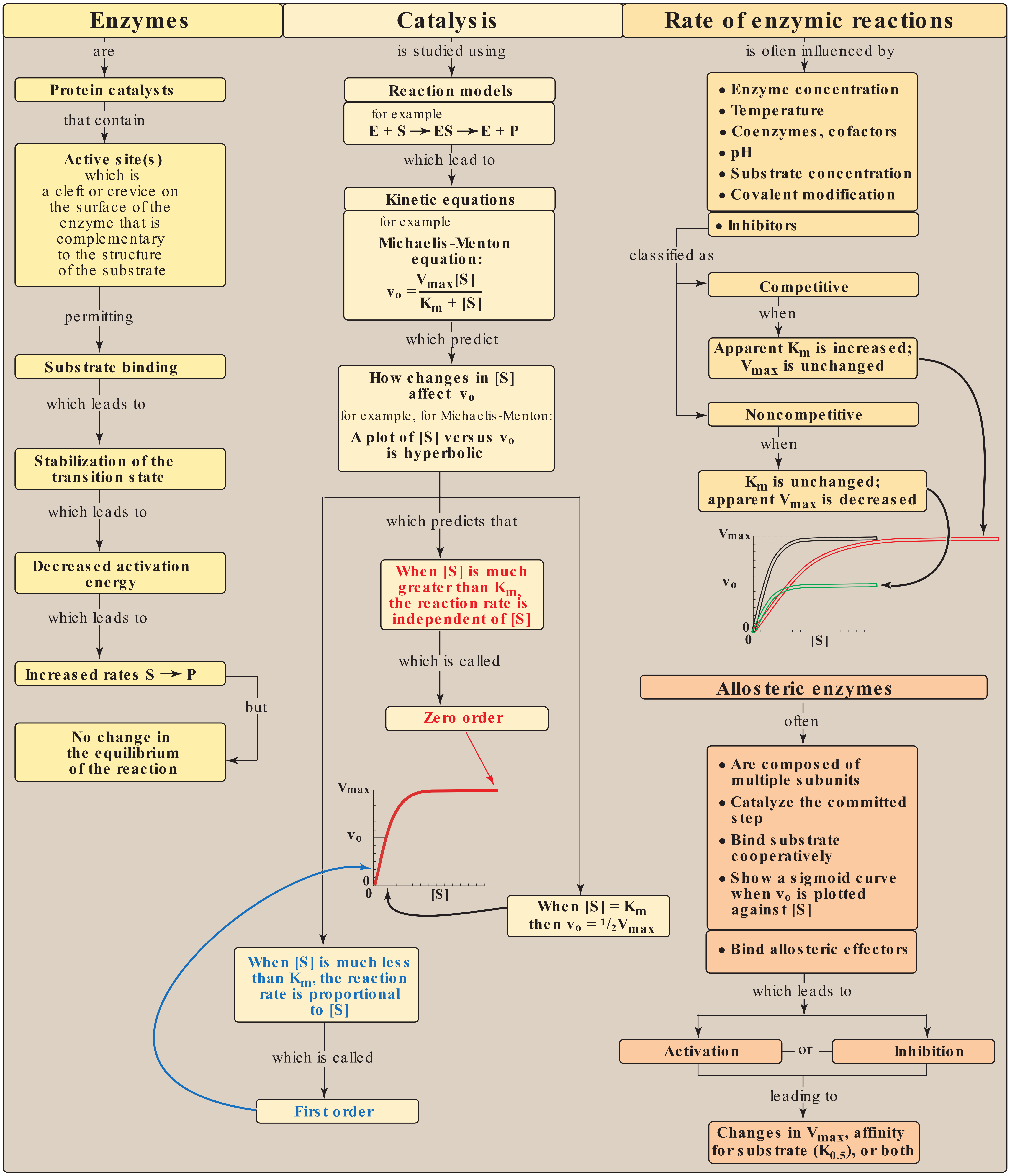

Enzymes
are
Protein catalysts
that contain
Active site(s) which is a cleft or crevice on the surface of the enzyme that is complementary to the structure of the substrate
permitting
Substrate binding
which leads to
Stabilization of the transition state
which leads to
Decreased activation energy
which leads to
Increased rates S → P
but
No change in the equilibrium of the reaction
Catalysis
is studied using
Reaction models
for example
E + S → ES → E + P
which lead to
Kinetic equations
for example
Michaelis-Menton equation:
$v_o = \frac{V_{max}[S]}{K_m + [S]}$
which predict
How changes in [S] affect v_o
for example, for Michaelis-Menton:
A plot of [S] versus v_o is hyperbolic
which predicts that
When [S] is much greater than K_m, the reaction rate is independent of [S]
which is called
Zero order
When [S] = K_m then v_o = $^1/_2 V_{max}$
When [S] is much less than K_m, the reaction rate is proportional to [S]
which is called
First order
V_{max}
v_o
0
0
[S]
Rate of enzymic reactions
is often influenced by
• Enzyme concentration
• Temperature
• Coenzymes, cofactors
• pH
• Substrate concentration
• Covalent modification
• Inhibitors
classified as
Competitive
when
Apparent K_m is increased; V_{max} is unchanged
Noncompetitive
when
K_m is unchanged; apparent V_{max} is decreased
V_{max}
v_o
0
0
[S]
Allosteric enzymes
often
• Are composed of multiple subunits
• Catalyze the committed step
• Bind substrate cooperatively
• Show a sigmoid curve when v_o is plotted against [S]
• Bind allosteric effectors
which leads to
Activation
or
Inhibition
leading to
Changes in V_{max}, affinity for substrate ($K_{0.5}$), or both

Figure 5.23
Key concept map for the enzymes. S = substrate; [S] = substrate concentration; P = product; E = enzyme; v_o = initial velocity; V_{max} = maximal velocity; K_m = Michaelis constant; $K_{0.5}$ = substrate concentration which gives half maximal velocity.

UNIT II:
Bioenergetics and Carbohydrate Metabolism

Bioenergetics and Oxidative Phosphorylation

6

I. OVERVIEW

Bioenergetics describes the transfer and utilization of energy in biologic systems. It makes use of a few basic ideas from the field of thermodynamics, particularly the concept of free energy. Changes in free energy provide a measure of the energetic feasibility of a chemical reaction and can, therefore, allow prediction of whether a reaction or process can take place. Bioenergetics concerns only the initial and final energy states of reaction components, not the mechanism or how much time is needed for the chemical change to take place (the rate). In short, bioenergetics predicts if a process is possible, whereas kinetics measures how fast the reaction occurs (see p. 54).

II. FREE ENERGY

The direction and extent to which a chemical reaction proceeds is determined by the degree to which two factors change during the reaction. These are enthalpy (ΔH, a measure of the change in heat content of the reactants and products) and entropy (ΔS, a measure of the change in randomness or disorder of reactants and products; Figure 6.1). Neither of these thermodynamic quantities by itself is sufficient to determine whether a chemical reaction will proceed spontaneously in the direction it is written. However, when combined mathematically (see Figure 6.1), enthalpy and entropy can be used to define a third quantity, free energy (G), which predicts the direction in which a reaction will spontaneously proceed.

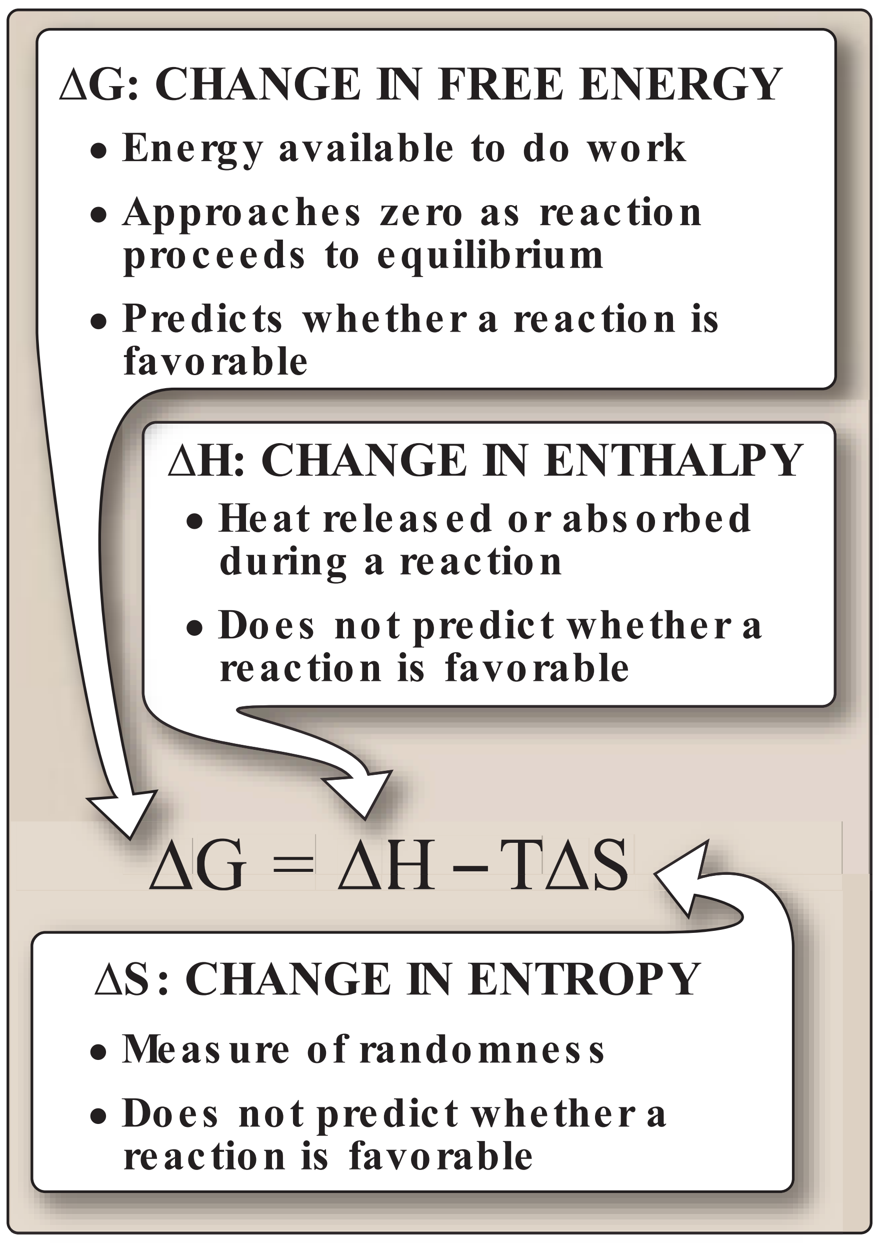

ΔG: CHANGE IN FREE ENERGY
• Energy available to do work
• Approaches zero as reaction proceeds to equilibrium
• Predicts whether a reaction is favorable
ΔH: CHANGE IN ENTHALPY
• Heat released or absorbed during a reaction
• Does not predict whether a reaction is favorable
ΔG = ΔH – TΔS
ΔS: CHANGE IN ENTROPY
• Measure of randomness
• Does not predict whether a reaction is favorable

Figure 6.1
Relationship between changes in free energy (G), enthalpy (H), and entropy (S). T is the absolute temperature in Kelvin (K): K = ^{o}C + 273.

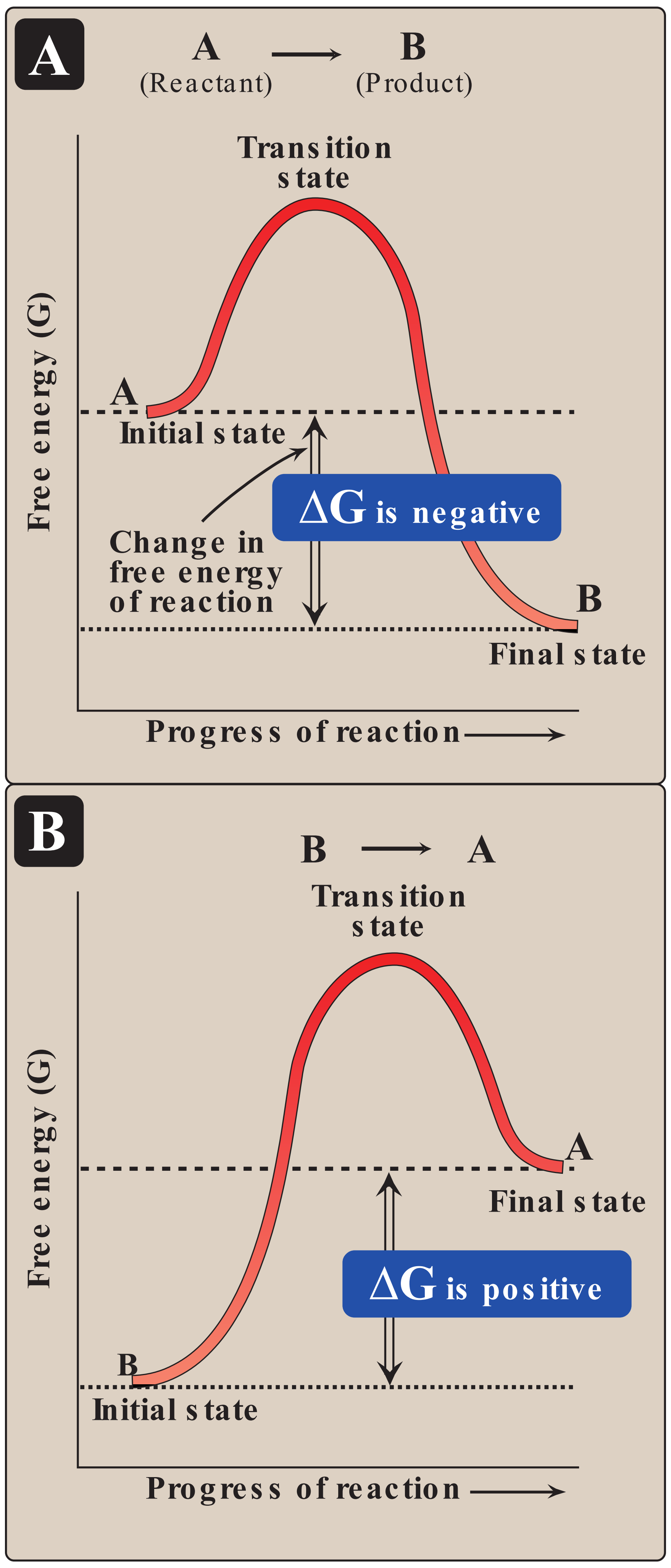


Figure 6.2
Change in free energy (ΔG) during a reaction. **A** The product has a lower free energy (G) than the reactant. **B** The product has a higher free energy than the reactant.

III. FREE ENERGY CHANGE

The change in free energy is represented in two ways, ΔG and ΔG^o. The first, ΔG (without the superscript "o"), represents the change in free energy and, thus, the direction of a reaction at any specified concentration of products and reactants. ΔG, then, is a variable. This contrasts with the standard free energy change, ΔG^o (with the superscript "o"), which is the energy change when reactants and products are at a concentration of 1 mol/l. [Note: The concentration of protons is assumed to be 10^{-7} mol/l (that is, pH = 7). This may be shown by a prime sign ('), for example, $\Delta G^{o\prime}$.] Although ΔG^o, a constant, represents energy changes at these nonphysiologic concentrations of reactants and products, it is nonetheless useful in comparing the energy changes of different reactions. Furthermore, ΔG^o can readily be determined from measurement of the equilibrium constant (see p. 72). This section outlines the uses of ΔG, and ΔG^o is described on p. 71.

A. Sign of ΔG and the direction of a reaction

ΔG can be used to predict the direction of a reaction at constant temperature and pressure. Consider the reaction:

$$A \rightleftarrows B$$

1. **Negative ΔG:** If ΔG is negative, there is a net loss of energy, and the reaction goes spontaneously as written (that is, A is converted into B) as shown in Figure 6.2A. The reaction is said to be exergonic.
2. **Positive ΔG:** If ΔG is positive, there is a net gain of energy, and the reaction does not go spontaneously from B to A (see Figure 6.2B). Energy must be added to the system to make the reaction go from B to A. The reaction is said to be endergonic.
3. **ΔG is zero:** If $\Delta G = 0$, the reactants are in equilibrium. [Note: When a reaction is proceeding spontaneously (that is, free energy is being lost) then the reaction continues until ΔG reaches zero and equilibrium is established.]

B. ΔG of the forward and back reactions

The free energy of the forward reaction (A → B) is equal in magnitude but opposite in sign to that of the back reaction (B → A). For example, if ΔG of the forward reaction is −5 kcal/mol, then that of the back reaction is +5 kcal/mol. [Note: ΔG can also be expressed in kilojoules per mole or kJ/mol (1 kcal = 4.2 kJ).]

C. ΔG and the concentration of reactants and products

The ΔG of the reaction A → B depends on the concentration of the reactant and product. At constant temperature and pressure, the following relationship can be derived:

$$\Delta G = \Delta G^o + RT \ln \frac{[B]}{[A]}$$

where ΔG^o is the standard free energy change (see below)
R is the gas constant (1.987 cal/mol K)
T is the absolute temperature (K)
[A] and [B] are the actual concentrations of the reactant and product
ln represents the natural logarithm

A reaction with a positive ΔG^o can proceed in the forward direction (have a negative overall ΔG) if the ratio of products to reactants ([B]/[A]) is sufficiently small (that is, the ratio of reactants to products is large). For example, consider the reaction:

$$\text{Glucose 6-phosphate} \rightleftarrows \text{fructose 6-phosphate}$$

Figure 6.3A shows reaction conditions in which the concentration of reactant, glucose 6-phosphate, is high compared with the concentration of product, fructose 6-phosphate. This means that the ratio of the product to reactant is small, and RT ln([fructose 6-phosphate]/[glucose 6-phosphate]) is large and negative, causing ΔG to be negative despite ΔG^o being positive. Thus, the reaction can proceed in the forward direction.

D. Standard free energy change

The standard free energy change, ΔG^o, is so called because it is equal to the free energy change, ΔG, under standard conditions (that is, when reactants and products are at 1 mol/l concentrations; see Figure 6.3B). Under these conditions, the natural logarithm of the ratio of products to reactants is zero (ln1 = 0), and, therefore, the equation shown at the bottom of the previous page becomes:

$$G = \Delta G^o + 0$$

1. **ΔG^o and the direction of a reaction:** Under standard conditions, ΔG^o can be used to predict the direction a reaction proceeds because, under these conditions, ΔG^o is equal to ΔG. However, ΔG^o cannot predict the direction of a reaction under physiologic conditions, because it is composed solely of constants (R, T, and K_{eq} [see below]) and is not, therefore, altered by changes in product or substrate concentrations.

2. **Relationship between ΔG^o and K_{eq}:** In a reaction A ⇄ B, a point of equilibrium is reached at which no further net chemical change takes place (that is, when A is being converted to B as fast as B is being converted to A). In this state, the ratio of [B] to [A] is constant, regardless of the actual concentrations of the two compounds:

$$K_{eq} = \frac{[B]_{eq}}{[A]_{eq}}$$

where K_{eq} is the equilibrium constant, and $[A]_{eq}$ and $[B]_{eq}$ are the concentrations of A and B at equilibrium. If the reaction A ⇄ B is allowed to go to equilibrium at constant temperature and pressure, then, at equilibrium, the overall ΔG is zero. Therefore,

$$\Delta G = 0 = \Delta G^o + RT \ln \frac{[B]_{eq}}{[A]_{eq}}$$

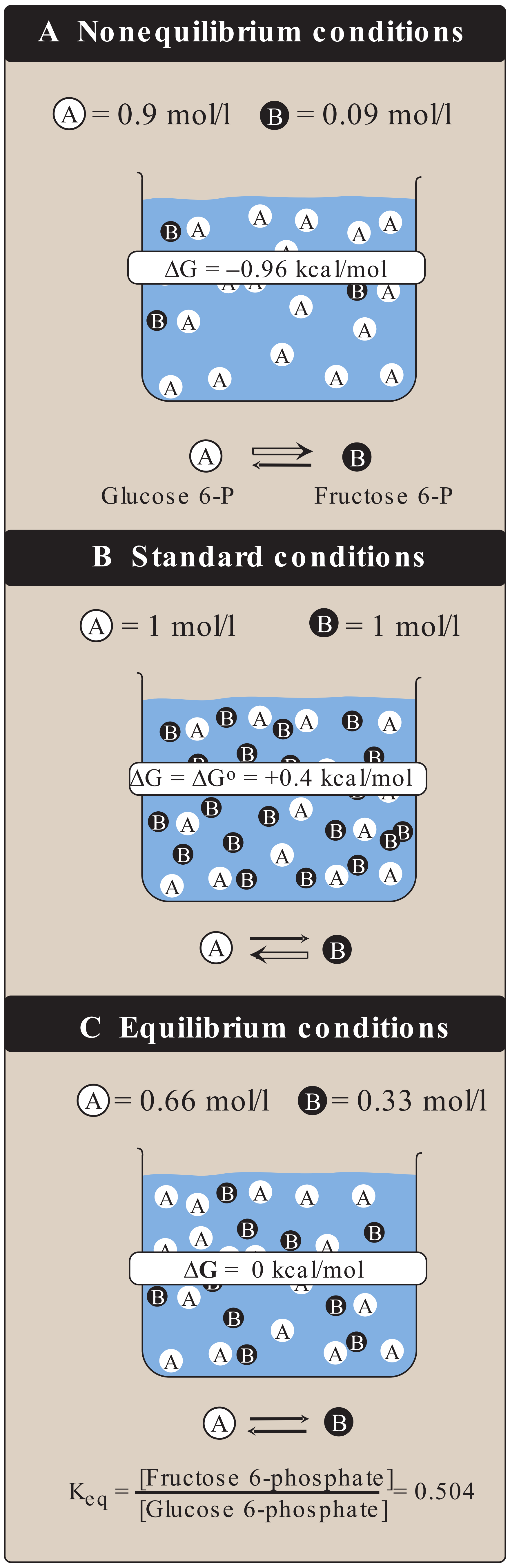


Figure 6.3
Free energy change (ΔG) of a reaction depends on the concentration of reactant Ⓐ and product Ⓑ. For the conversion of glucose 6-phosphate to fructose 6-phosphate, ΔG is negative when the ratio of reactant Ⓐ to product Ⓑ is large (top, panel A), is positive under standard conditions (middle, panel B), and is zero at equilibrium (bottom, panel C). ΔG^0 = standard free energy change.

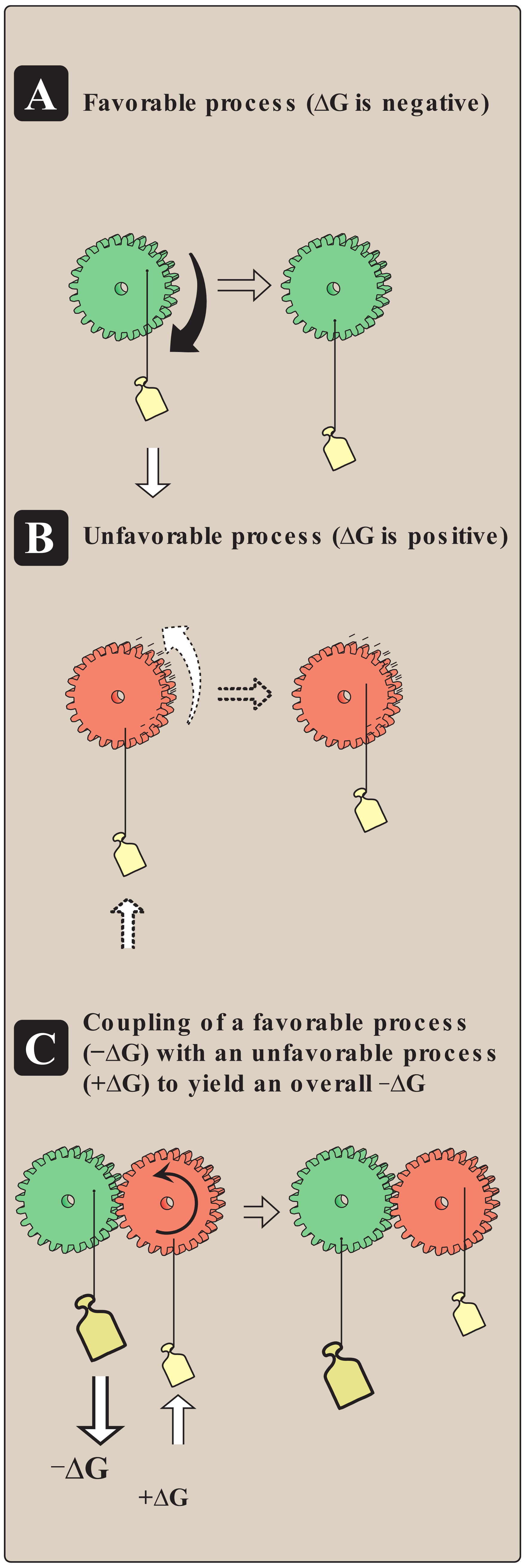

A Favorable process (ΔG is negative)
B Unfavorable process (ΔG is positive)
C Coupling of a favorable process (−ΔG) with an unfavorable process (+ΔG) to yield an overall −ΔG
−ΔG
+ΔG

Figure 6.4
Mechanical model of coupling of favorable and unfavorable processes. **A** Gear with weight attached spontaneously turns in the direction that achieves the lowest energy state. **B** The reverse movement is energetically unfavorable (not spontaneous). **C** The energetically favorable movement can drive the unfavorable one.

where the actual concentrations of A and B are equal to the equilibrium concentrations of reactant and product $[A]_{eq}$ and $[B]_{eq}$, and their ratio is equal to the K_{eq}. Thus,

$$\boxed{\Delta G^o = -RT \ln K_{eq}}$$

This equation allows some simple predictions:

If $K_{eq} = 1$, then $\Delta G^o = 0$	$A \rightleftarrows B$
If $K_{eq} > 1$, then $\Delta G^o < 0$	$A \overset{\longrightarrow}{\leftarrow} B$
If $K_{eq} < 1$, then $\Delta G^o > 0$	$A \overset{\rightarrow}{\longleftarrow} B$

3. ΔG^o of two consecutive reactions: The ΔG^os are additive in any sequence of consecutive reactions, as are the ΔGs. For example:

Glucose + ATP	→ glucose 6-phosphate + ADP	$\Delta G^o =$	–4,000 cal/mol
Glucose 6-phosphate	→ fructose 6-phosphate	$\Delta G^o =$	+400 cal/mol
Glucose + ATP	→ fructose 6-phosphate + ADP	$\Delta G^o =$	–3,600 cal/mol

4. ΔGs of a pathway: The additive property of free energy changes is very important in biochemical pathways through which substrates must pass in a particular direction (for example, A → B → C → D →...). As long as the sum of the ΔGs of the individual reactions is negative, the pathway can potentially proceed as written, even if some of the individual reactions of the pathway have a positive ΔG. The actual rate of the reactions does, of course, depend on the lowering of activation energies by the enzymes that catalyze the reactions (see p. 55).

IV. ADENOSINE TRIPHOSPHATE AS AN ENERGY CARRIER

Reactions or processes that have a large positive ΔG, such as moving ions against a concentration gradient across a cell membrane, are made possible by coupling the endergonic movement of ions with a second, spontaneous process with a large negative ΔG such as the exergonic hydrolysis of adenosine triphosphate (ATP). [Note: In the absence of enzymes, ATP is a stable molecule because its hydrolysis has a high activation energy (see p. 55).] Figure 6.4 shows a mechanical model of energy coupling. The simplest example of energy coupling in biologic reactions occurs when the energy-requiring and the energy-yielding reactions share a common intermediate.

A. Common intermediates

Two chemical reactions have a common intermediate when they occur sequentially so that the product of the first reaction is a substrate for the second. For example, given the reactions

$$A + B \rightarrow C + D$$
$$D + X \rightarrow Y + Z$$

D is the common intermediate and can serve as a carrier of chemical energy between the two reactions. Many coupled reactions use ATP to generate a common intermediate. These reactions may involve the transfer of a phosphate group from ATP to another molecule. Other reactions involve the transfer of phosphate from an energy-rich intermediate to adenosine diphosphate (ADP), forming ATP.

B. Energy carried by adenosine triphosphate

ATP consists of a molecule of adenosine (adenine + ribose) to which three phosphate groups are attached (Figure 6.5). If one phosphate is removed, ADP is produced. If two phosphates are removed, adenosine monophosphate (AMP) results. The standard free energy of hydrolysis of ATP, ΔG^o, is approximately –7.3 kcal/mol for each of the two terminal phosphate groups. Because of this large negative ΔG^o, ATP is called a high-energy phosphate compound.

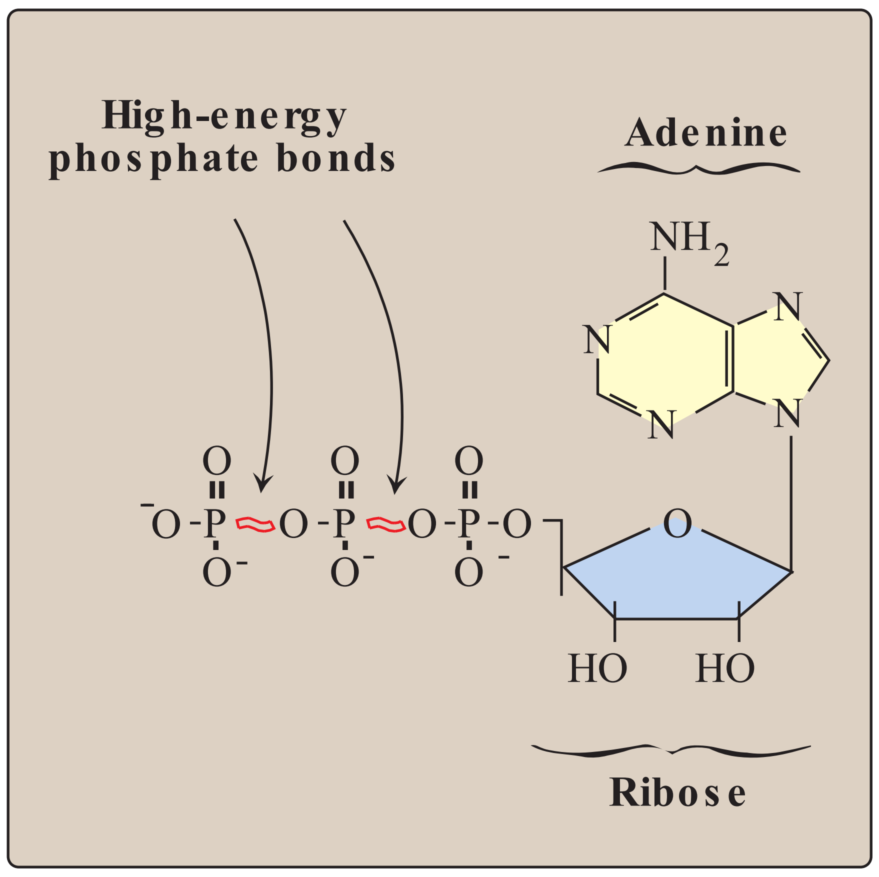


Figure 6.5
Adenosine triphosphate.

V. ELECTRON TRANSPORT CHAIN

Energy-rich molecules, such as glucose, are metabolized by a series of oxidation reactions ultimately yielding CO_2 and water (Figure 6.6). The metabolic intermediates of these reactions donate electrons to specific coenzymes, nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD), to form the energy-rich reduced forms, NADH and $FADH_2$. These reduced coenzymes can, in turn, each donate a pair of electrons to a specialized set of electron carriers, collectively called the electron transport chain (ETC), described in this section. As electrons are passed down the ETC, they lose much of their free energy. This energy is used to move protons across the inner mitochondrial membrane, creating a proton gradient that drives the production of ATP from ADP and inorganic phosphate (P_i), described on p. 77. The coupling of electron transport with ATP synthesis is called oxidative phosphorylation, often denoted as OXPHOS. It proceeds continuously in all tissues that contain mitochondria. [Note: The remainder of the free energy not trapped as ATP is used to drive ancillary reactions such as calcium transport into mitochondria (see p. 133) and to generate heat.]

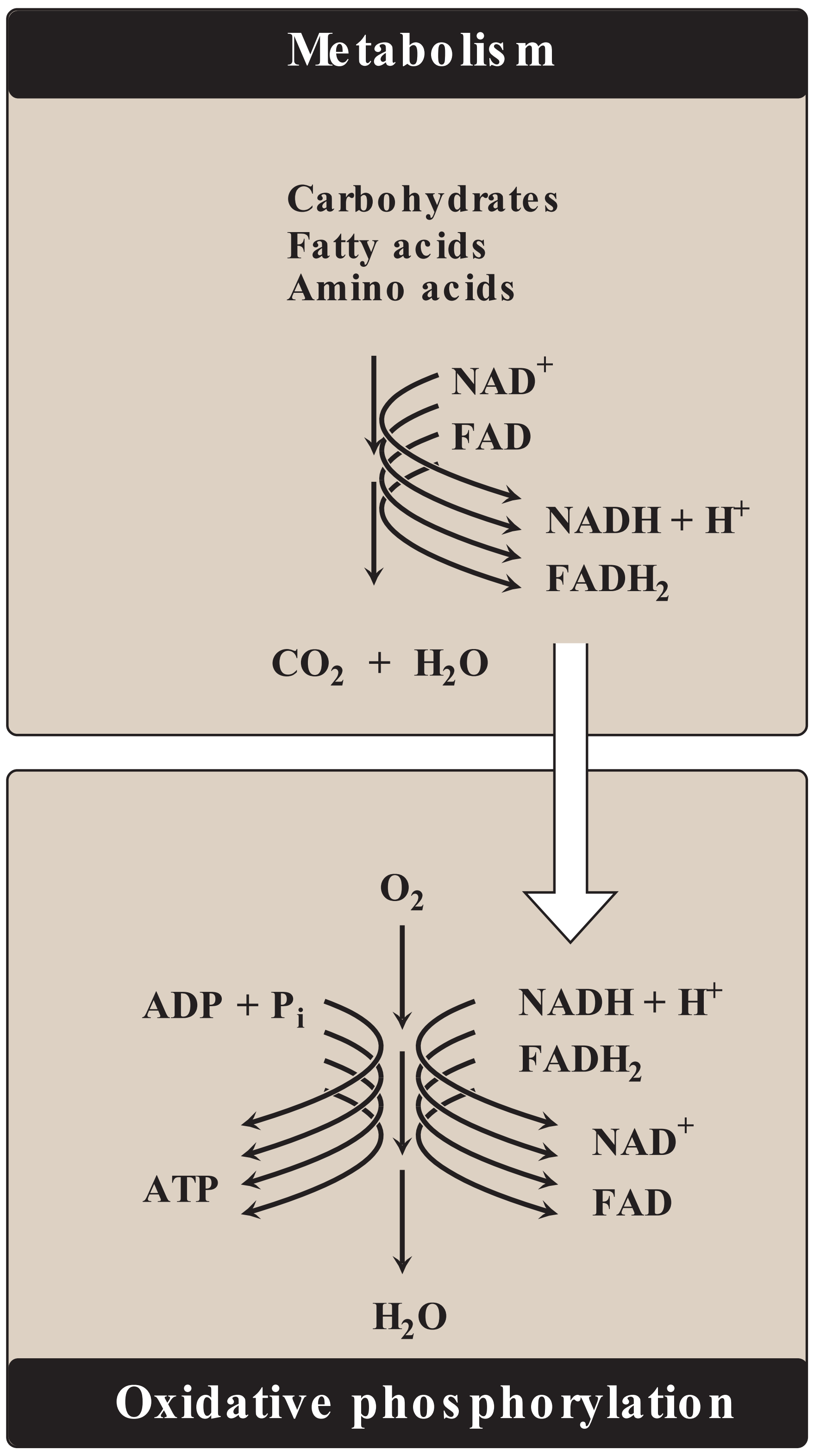


Figure 6.6
The metabolic breakdown of energy-yielding molecules. NAD(H) = nicotinamide adenine dinucleotide; $FAD(H_2)$= flavin adenine dinucleotide; ADP = adenosine diphosphate; ATP = adenosine triphosphate; P_i = inorganic phosphate.

A. The electron transport chain of the mitochondrion

The ETC (except for cytochrome c; see p. 75) is located in the inner mitochondrial membrane and is the final common pathway by which electrons derived from different fuels of the body flow to oxygen (O_2).

1. **Membranes of the mitochondrion:** The mitochondrion contains an outer and an inner membrane separated by the intermembrane space. Although the outer membrane contains special channels (formed by the protein porin), making it freely permeable to most ions and small molecules, the inner membrane is a specialized structure that is impermeable to most small ions, including protons and small molecules such as ATP, ADP, pyruvate, and other metabolites important to mitochondrial function (Figure 6.7). Specialized carriers or transport systems are required to move ions or molecules across this membrane. The inner mitochondrial membrane is unusually rich in protein, over half of which is directly involved in oxidative phosphorylation. It also is highly convoluted. The convolutions, called cristae, serve to greatly increase the surface area of the inner membrane.

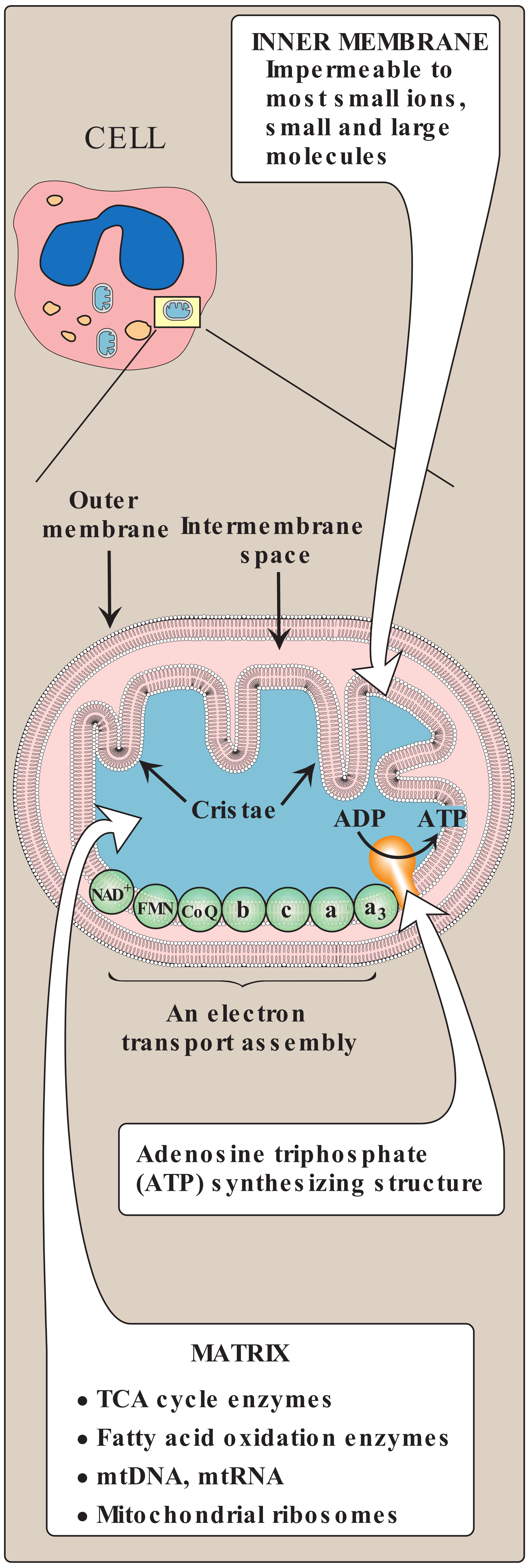

INNER MEMBRANE
Impermeable to most small ions, small and large molecules
CELL
Outer membrane
Intermembrane space
Cristae
ADP
ATP
NAD+
FMN
CoQ
b
c
a
a3
An electron transport assembly
Adenosine triphosphate (ATP) synthesizing structure
MATRIX
• TCA cycle enzymes
• Fatty acid oxidation enzymes
• mtDNA, mtRNA
• Mitochondrial ribosomes

Figure 6.7
Structure of a mitochondrion showing schematic representation of the electron transport chain and the ATP synthesizing structure on the inner membrane. [Note: In contrast to the inner membrane, the outer membrane is highly permeable, and the milieu of the intermembrane space is like that of the cytosol.] mtDNA = mitochondrial DNA; mtRNA = mitochondrial RNA; TCA = tricarboxylic acid.

2. **Matrix of the mitochondrion:** This gel-like solution in the interior of mitochondria is also rich in protein. These molecules include the enzymes responsible for the oxidation of pyruvate, amino acids, and fatty acids (by β-oxidation) as well as those of the tricarboxylic acid (TCA) cycle. The synthesis of glucose, urea, and heme occurs partially in the matrix of mitochondria. In addition, the matrix contains NAD^+ and FAD (the oxidized forms of the two coenzymes that are required as hydrogen acceptors), and ADP and P_i, which are used to produce ATP. [Note: The matrix also contains mitochondrial DNA (mtDNA) and RNA (mtRNA) and ribosomes.]

B. Organization of the electron transport chain

The inner mitochondrial membrane contains five separate protein complexes, called Complexes I, II, III, IV, and V. Complexes I–IV each contain part of the ETC (Figure 6.8). These complexes accept or donate electrons to the relatively mobile electron carriers, coenzyme Q and cytochrome c. Each carrier in the ETC can receive electrons from an electron donor and can subsequently donate electrons to the next acceptor in the chain. The electrons ultimately combine with O_2 and protons to form water. This requirement for O_2 makes the electron transport process the respiratory chain, which accounts for the greatest portion of the body's use of O_2. Complex V is described on p. 78.

C. Reactions of the electron transport chain

With the exception of coenzyme Q, which is a lipid-soluble quinone, all members of this chain are proteins. These may function as enzymes as is the case with the flavin-containing dehydrogenases, may contain iron as part of an iron-sulfur center, may contain iron as part of the porphyrin prosthetic group of heme as in the cytochromes, or may contain copper as does the cytochrome a + a_3 complex.

1. **Formation of NADH:** NAD^+ is reduced to NADH by dehydrogenases that remove two hydrogen atoms from their substrate. (For examples of these reactions, see the discussion of the dehydrogenases of the TCA cycle, p. 112.) Both electrons but only one proton (that is, a hydride ion [$:H^-$]) are transferred to the NAD^+, forming NADH plus a free proton.

2. **NADH dehydrogenase:** The free proton plus the hydride ion carried by NADH are transferred to NADH dehydrogenase, a protein complex (Complex I) embedded in the inner mitochondrial membrane. Complex I has a tightly bound molecule of flavin mononucleotide (FMN), a coenzyme structurally related to FAD (see Figure 28.15, p. 380) that accepts the two hydrogen atoms ($2e^- + 2H^+$), becoming $FMNH_2$. NADH dehydrogenase also contains peptide subunits with iron-sulfur centers (Figure 6.9). At Complex I, electrons move from NADH to FMN to the iron of the iron-sulfur centers and then to coenzyme Q. As electrons flow, they lose energy. This energy is used to pump protons across the inner mitochondrial membrane, from the matrix to the intermembrane space.

3. **Succinate dehydrogenase:** At Complex II, electrons from the succinate dehydrogenase–catalyzed oxidation of succinate to fumarate move from the coenzyme, $FADH_2$, to an iron-sulfur protein,

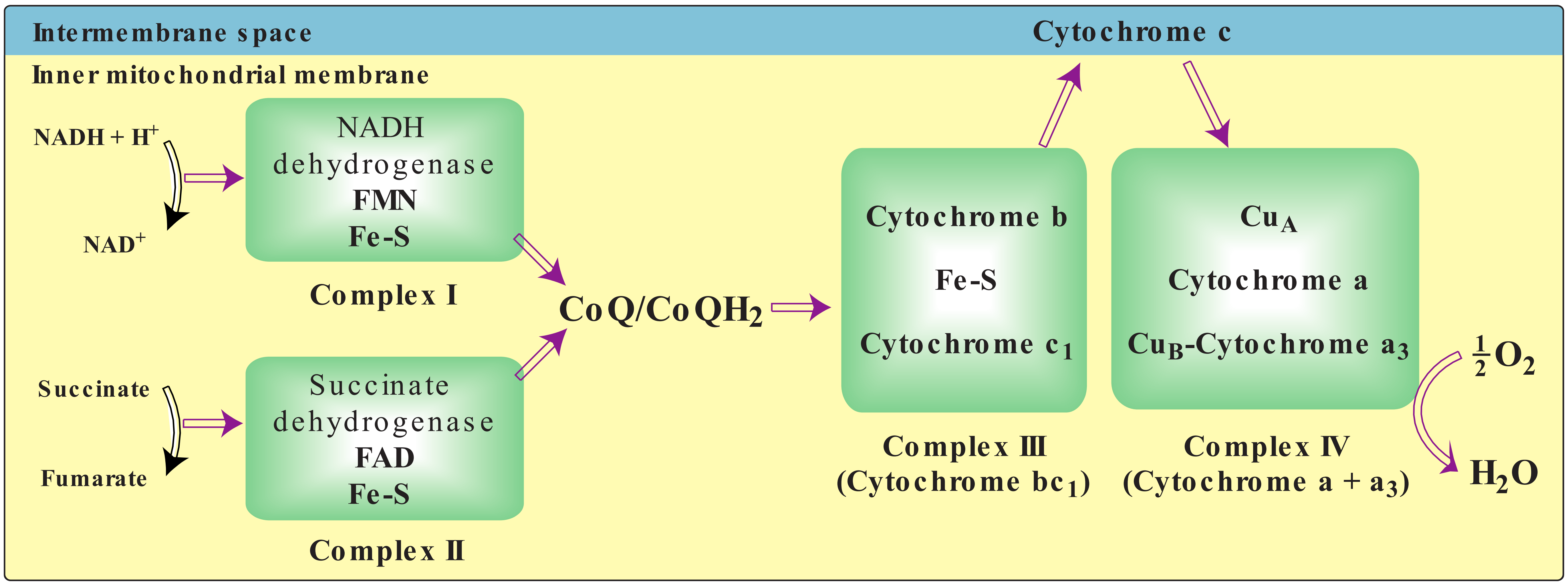

Intermembrane space
Cytochrome c
Inner mitochondrial membrane
NADH + H+
NAD+
NADH dehydrogenase
FMN
Fe-S
Complex I
Succinate
Fumarate
Succinate dehydrogenase
FAD
Fe-S
Complex II
CoQ/CoQH2
Cytochrome b
Fe-S
Cytochrome c1
Complex III
(Cytochrome bc1)
CuA
Cytochrome a
CuB-Cytochrome a3
Complex IV
(Cytochrome a + a3)
½O2
H2O

Figure 6.8
Electron transport chain. The flow of electrons is shown by the magenta arrows. NAD(H) = nicotinamide adenine dinucleotide; FMN = flavin mononucleotide; FAD = flavin adenine dinucleotide; Fe-S = iron-sulfur center; CoQ = coenzyme Q.

and then to coenzyme Q. [Note: No energy is lost in this process, and, therefore, no protons are pumped at Complex II.]

4. **Coenzyme Q:** Coenzyme Q (CoQ) is a quinone derivative with a long, hydrophobic isoprenoid tail. It is also called ubiquinone because it is ubiquitous in biologic systems. CoQ is a mobile electron carrier and can accept hydrogen atoms from NADH dehydrogenase (Complex I), from succinate dehydrogenase (Complex II), and from other mitochondrial dehydrogenases: glycerophosphate dehydrogenase (see p. 79) and acyl CoA dehydrogenase (see p. 192). CoQ transfers electrons to Complex III (cytochrome bc_1). CoQ, then, links the flavoprotein dehydrogenases to the cytochromes.

5. **Cytochromes:** The remaining members of the ETC are cytochrome proteins. Each contains a heme group (a porphyrin ring plus iron). Unlike the heme groups of hemoglobin, the cytochrome iron is reversibly converted from its ferric (Fe^{3+}) to its ferrous (Fe^{2+}) form as a normal part of its function as an acceptor and donor of electrons. Electrons are passed along the chain from cytochrome bc_1 (Complex III), to cytochrome c, and then to cytochromes a + a_3 (Complex IV; see Figure 6.8). As electrons flow, protons are pumped across the inner mitochondrial membrane at Complexes III and IV. [Note: Cytochrome c is located in the intermembrane space, loosely associated with the outer face of the inner membrane. As seen with CoQ, cytochrome c is a mobile carrier of electrons.]

6. **Cytochrome a + a_3:** This cytochrome complex (Complex IV) is the only electron carrier in which the heme iron has an available coordination site that can react directly with O_2 and so also is called cytochrome oxidase. At Complex IV, the transported electrons, O_2, and free protons are brought together, and O_2 is reduced to water (see Figure 6.8). [Note: Four electrons are required to reduce one molecule of O_2 to two molecules of water.] Cytochrome oxidase

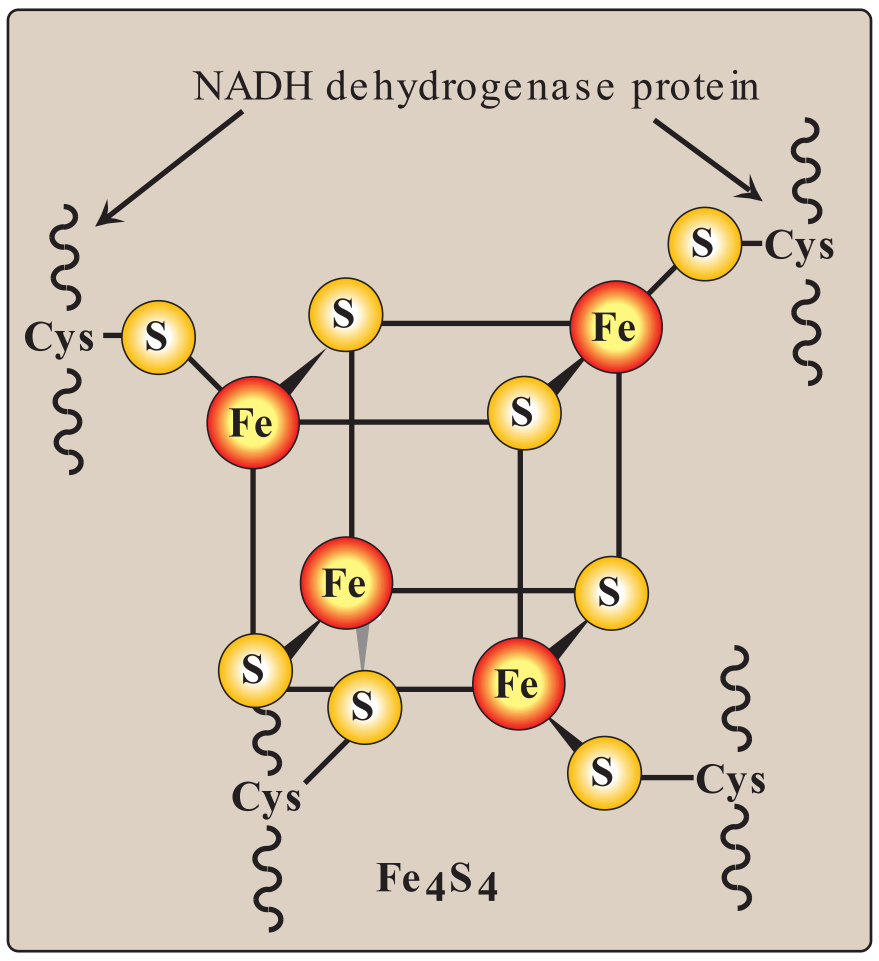

NADH dehydrogenase protein
Cys
S
Fe
Cys
S
Fe
S
S
Fe
S
S
Fe
S
Cys
Cys
S
S
Cys
Fe4S4

Figure 6.9
Iron-sulfur (Fe-S) center of Complex I. [Note: Complexes II and III also contain iron-sulfur centers.] NADH = nicotinamide adenine dinucleotide;Cys = cysteine.

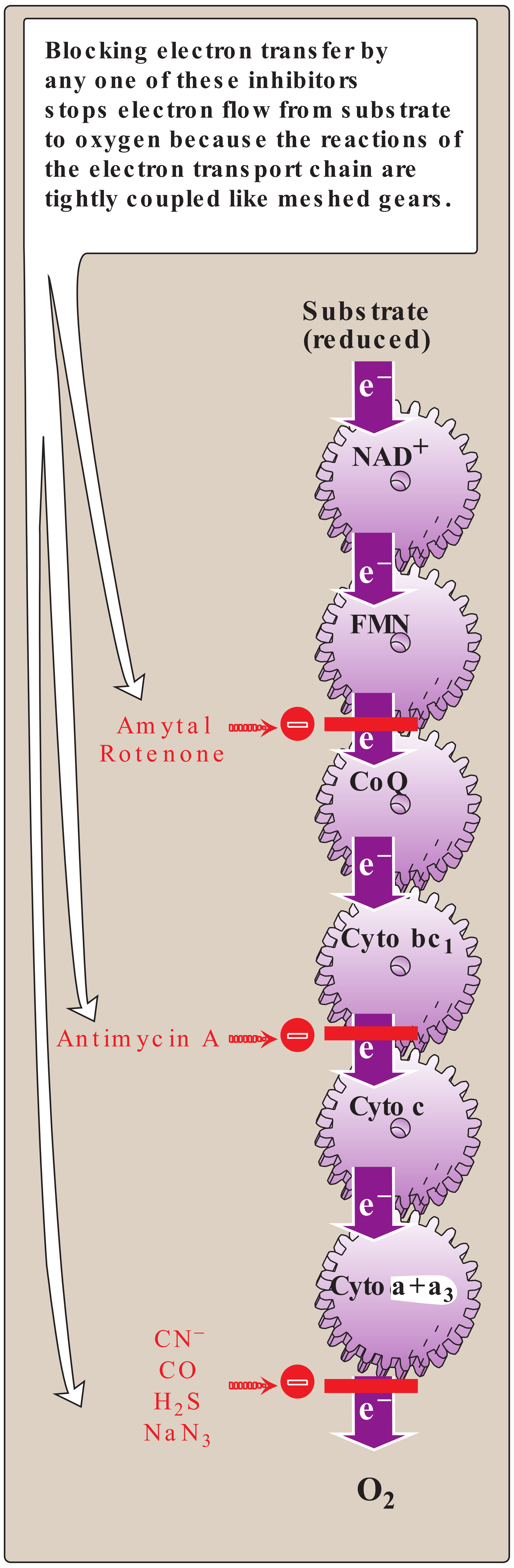

Blocking electron transfer by any one of these inhibitors stops electron flow from substrate to oxygen because the reactions of the electron transport chain are tightly coupled like meshed gears.

Figure 6.10
Site-specific inhibitors of electron transport shown using a mechanical model for the coupling of oxidation-reduction reactions. [Note: Figure illustrates normal direction of electron flow.] CN^- = cyanide; CO = carbon monoxide; H_2S = hydrogen sulfide; NaN_3 = sodium azide; FMN = flavin mononucleotide; FAD = flavin adenine dinucleotide; CoQ = coenzyme Q; Cyto = cytochrome.

contains copper (Cu) atoms that are required for this complicated reaction to occur. Electrons move from Cu_A to cytochrome a to cytochrome a_3 (in association with Cu_B) to O_2.

7. **Site-specific inhibitors:** Site-specific inhibitors of electron transport have been identified and are illustrated in Figure 6.10. These compounds prevent the passage of electrons by binding to a component of the chain, blocking the oxidation-reduction reaction. Therefore, all electron carriers before the block are fully reduced, whereas those located after the block are oxidized. [Note: Inhibition of electron transport inhibits ATP synthesis because these processes are tightly coupled (see p. 77).]

Incomplete reduction of oxygen to water produces reactive oxygen species (ROS), such as superoxide ($O_2^{-}\bullet$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH•). ROS damage DNA and proteins and cause lipid peroxidation. Enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase are cellular defenses against ROS.

D. Release of free energy during electron transport

The free energy released as electrons are transferred along the ETC from an electron donor (reducing agent or reductant) to an electron acceptor (oxidizing agent or oxidant) is used to pump protons at Complexes I, III, and IV. [Note: The electrons can be transferred as hydride ions ($:H^-$) to NAD^+; as hydrogen atoms (•H) to FMN, CoQ, and FAD; or as electrons (e^-) to cytochromes.]

1. **Redox pairs:** Oxidation (loss of electrons) of one substance is always accompanied by reduction (gain of electrons) of a second. For example, Figure 6.11 shows the oxidation of NADH to NAD^+ by NADH dehydrogenase at Complex I, accompanied by the reduction of FMN, the prosthetic group, to $FMNH_2$. Such oxidation-reduction reactions can be written as the sum of two separate half-reactions, one an oxidation and the other a reduction (see Figure 6.11). NAD^+ and NADH form a redox pair, as do FMN and $FMNH_2$. Redox pairs differ in their tendency to lose electrons. This tendency is a characteristic of a particular redox pair and can be quantitatively specified by a constant, E_o (the standard reduction potential), with units in volts.

2. **Standard reduction potential:** The E_o of various redox pairs can be ordered from the most negative E_o to the most positive. The more negative the E_o of a redox pair, the greater the tendency of the reductant member of that pair to lose electrons. The more positive the E_o, the greater the tendency of the oxidant member of that pair to accept electrons. Therefore, electrons flow from the pair with the more negative E_o to that with the more positive E_o. The E_o values for some members of the ETC are shown in Figure 6.12. [Note: The components of the chain are arranged in order of increasingly positive E_o values.]

3. **Relationship of ΔG^o to ΔE_o:** The ΔG^o is related directly to the magnitude of the change in E_o:

$$G^o = -\Delta n\ F\ \Delta E_o$$

where n = number of electrons transferred (1 for a cytochrome, 2 for NADH, $FADH_2$, and coenzyme Q)
F = Faraday constant (23.1 kcal/volt mol)
$E_o = E_o\Delta$of the electron-accepting pair minus the E_o of the electron-donating pair
ΔG^o = change in the standard free energy

4. **ΔG^o of ATP:** The ΔG^o for the phosphorylation of ADP to ATP is +7.3 kcal/mol. The transport of a pair of electrons from NADH to O_2 through the ETC releases 52.58 kcal. Therefore, more than sufficient energy is available to produce 3 ATP from 3 ADP and 3 P_i (3 x 7.3 = 21.9 kcal/mol), sometimes expressed as a P:O ratio (ATP made per O atom reduced) of 3:1. The remaining calories are used for ancillary reactions or released as heat. [Note: The P:O for $FADH_2$ is 2:1 because Complex I is bypassed.]

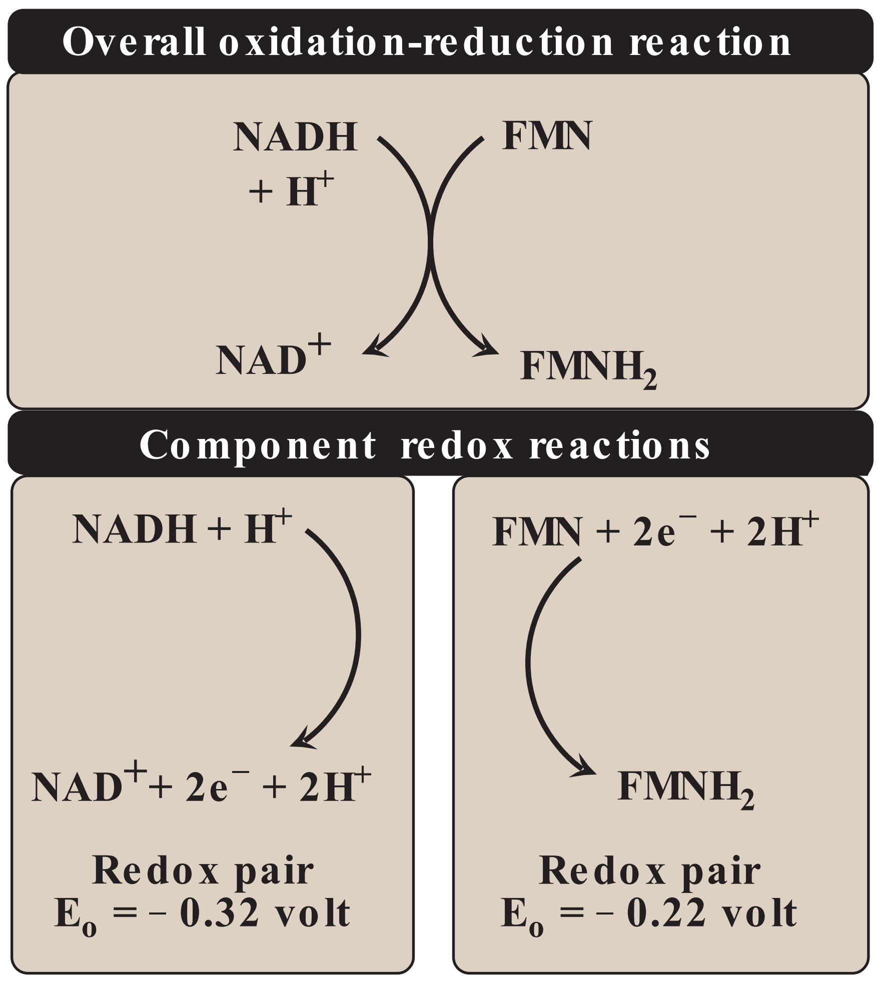


Figure 6.11
Oxidation of NADH by FMN, separated into two component half-reactions. NAD(H) = nicotinamide adenine dinucleotide; $FMN(H_2)$ = flavin mononucleotide.

VI. PHOSPHORYLATION OF ADP TO ATP

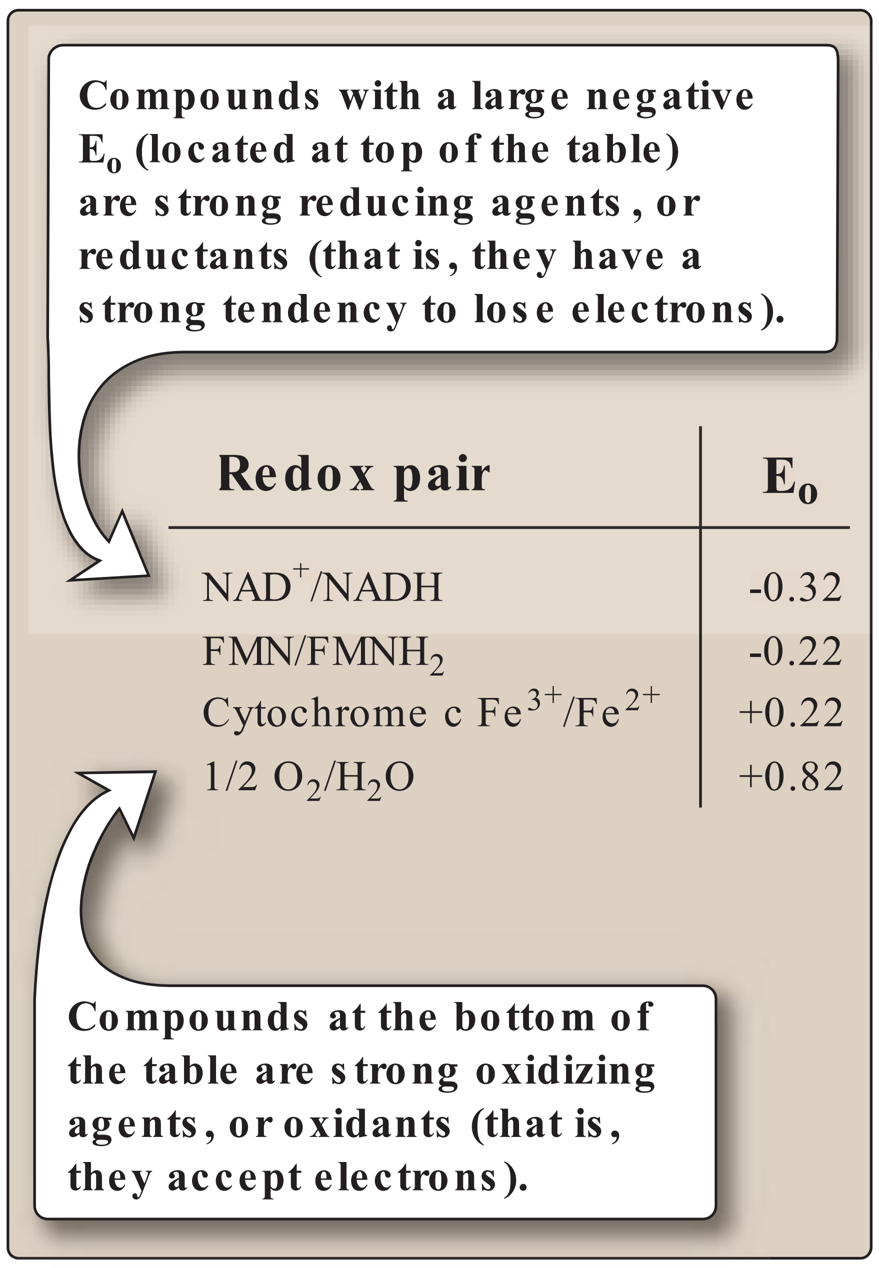
The transfer of electrons down the ETC is energetically favored because NADH is a strong electron donor and O_2 is an avid electron acceptor. However, the flow of electrons does not directly result in ATP synthesis.

A. Chemiosmotic hypothesis

The chemiosmotic hypothesis (also known as the Mitchell hypothesis) explains how the free energy generated by the transport of electrons by the ETC is used to produce ATP from ADP + P_i.

1. **Proton pump:** Electron transport is coupled to the phosphorylation of ADP by the pumping of protons across the inner mitochondrial membrane, from the matrix to the intermembrane space, at Complexes I, III, and IV. This process creates an electrical gradient (with more positive charges on the outside of the membrane than on the inside) and a pH gradient (the outside of the membrane is at a lower pH than the inside) as shown in Figure 6.13. The energy generated by this proton gradient is sufficient to drive ATP synthesis. Thus, the proton gradient serves as the common intermediate that couples oxidation to phosphorylation.

2. **ATP synthase:** The multisubunit enzyme ATP synthase (Complex V; see Figure 6.14) synthesizes ATP using the energy of the proton gradient. It contains a domain (F_o) that spans the inner mitochondrial membrane, and an extramembranous domain (F_1) that appears as a sphere that protrudes into the mitochondrial matrix (see Figure 6.13). The chemiosmotic hypothesis proposes that after protons have been pumped to the cytosolic side of the inner mitochondrial membrane, they reenter the matrix by passing through a proton channel in the F_o domain, driving rotation of the c ring of F_o and, at the same time, dissipating the pH and electrical gradients. F_o rotation causes conformational changes in the β subunits of the F_1 domain that allow them to bind ADP + P_i, phosphorylate ADP to ATP, and release ATP. [Note: ATP synthase is also called F_1/F_o-ATPase because the isolated enzyme can catalyze the hydrolysis of ATP to ADP and P_i.]



Compounds with a large negative E_o (located at top of the table) are strong reducing agents, or reductants (that is, they have a strong tendency to lose electrons).

Redox pair	E_o
$NAD^+/NADH$	-0.32
$FMN/FMNH_2$	-0.22
Cytochrome c Fe^{3+}/Fe^{2+}	+0.22
1/2 O_2/H_2O	+0.82

Compounds at the bottom of the table are strong oxidizing agents, or oxidants (that is, they accept electrons).

Figure 6.12
Standard reduction potentials (E_o) of some reactions. NAD(H) = nicotinamide adenine dinucleotide; $FMN(H_2)$ = flavin mononucleotide.

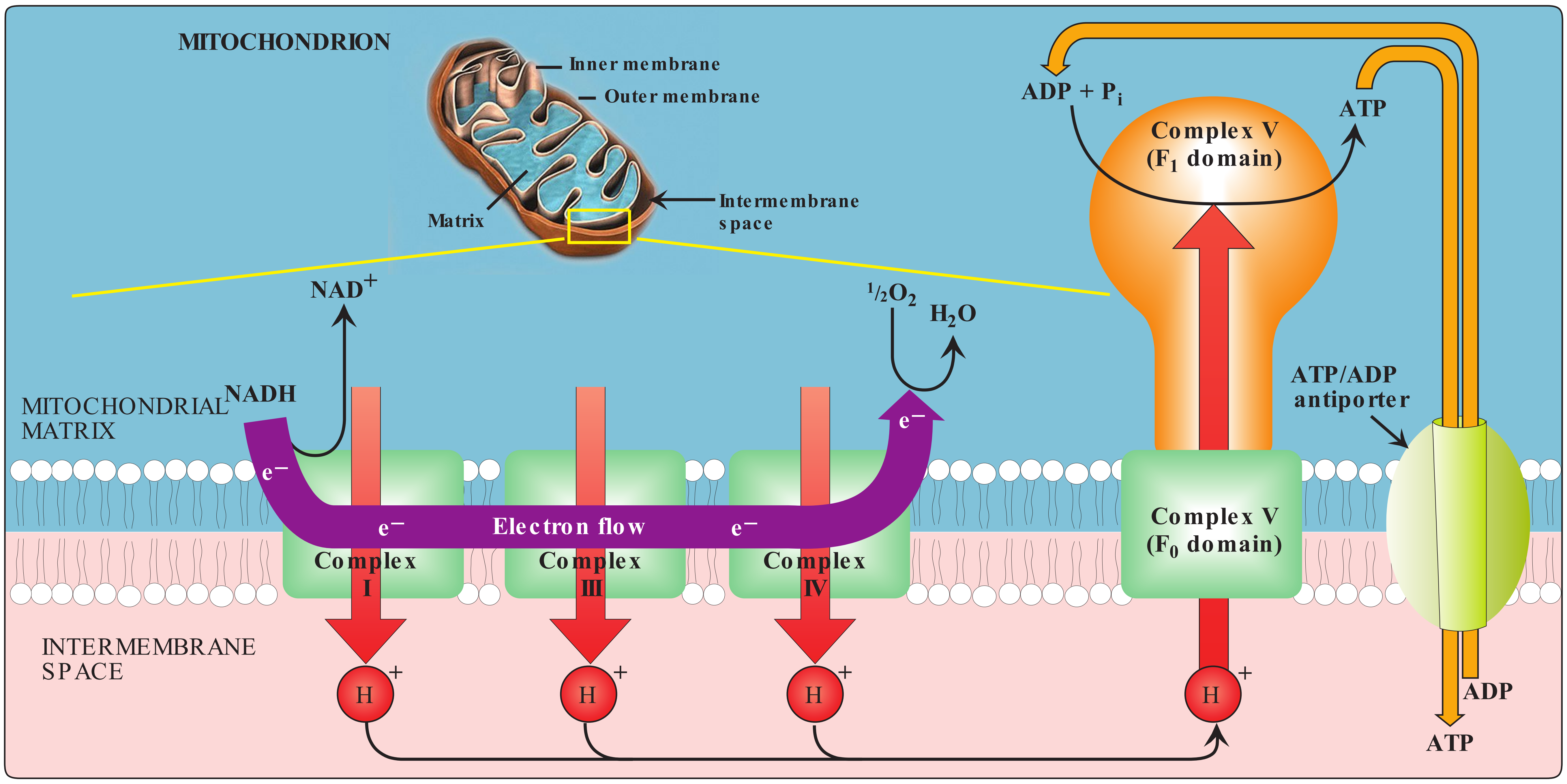

MITOCHONDRION
Inner membrane
Outer membrane
Matrix
Intermembrane space
NAD+
NADH
MITOCHONDRIAL MATRIX
e−
Electron flow
Complex I
Complex III
Complex IV
½O2
H2O
INTERMEMBRANE SPACE
H+
ADP + Pi
ATP
Complex V (F1 domain)
Complex V (F0 domain)
ATP/ADP antiporter
ADP
ATP

Figure 6.13
Electron transport chain shown in association with the transport of protons (H^+). A total of ten H^+ are pumped for each nicotinamide adenine dinucleotide (NADH) oxidized. [Note: H^+ are not pumped at Complex II.]

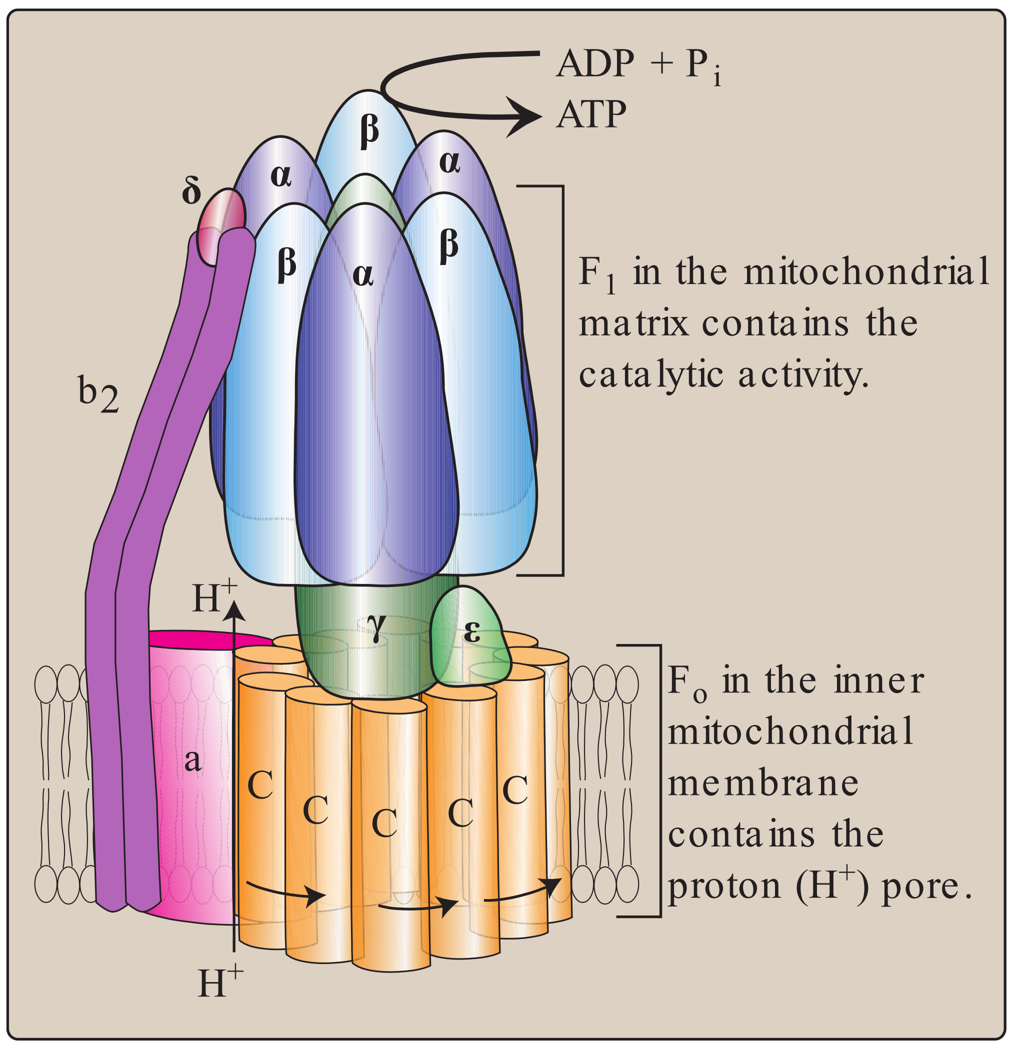

ADP + Pi
ATP
β
α
α
δ
β
α
β
b2
F1 in the mitochondrial matrix contains the catalytic activity.
H+
γ
ε
a
C
C
C
C
C
Fo in the inner mitochondrial membrane contains the proton (H+) pore.
H+

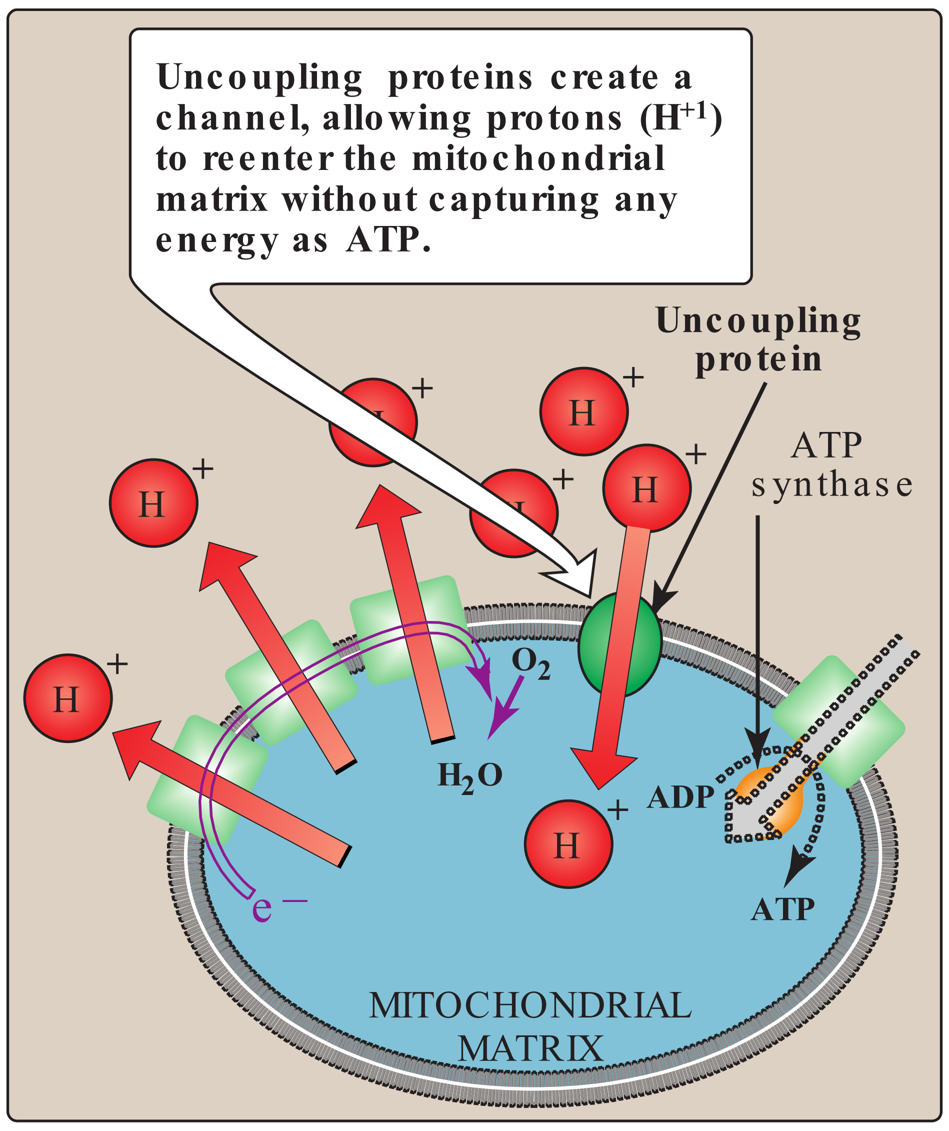
Figure 6.14
ATP synthase (F_1F_o–ATPase). [Note: The rotation of the ring of c subunits in the F_o domain results in conformational changes in the β subunits of the F_1 domain that allow phosphorylation of adenosine diphosphate (ADP) to adenosine triphosphate (ATP). P_i = inorganic phosphate.

a. Coupling in oxidative phosphorylation: In normal mitochondria, ATP synthesis is coupled to electron transport through the proton gradient. Increasing (or decreasing) one process has the same effect on the other. For example, hydrolysis of ATP to ADP and P_i in energy-requiring reactions increases the availability of substrates for ATP synthase and, thus, increases proton flow through the enzyme. Electron transport and proton pumping by the ETC increase to maintain the proton gradient. [Note: Increased oxidation of NADH at Complex I and, consequently, an increase in NADH-producing pathways of metabolism, such as the TCA cycle, results.]

b. Oligomycin: This drug binds to the F_o (hence the letter "o") domain of ATP synthase, closing the proton channel and preventing reentry of protons into the matrix, thereby preventing phosphorylation of ADP to ATP. Because the pH and electrical gradients cannot be dissipated in the presence of this drug, electron transport stops because of the difficulty of pumping any more protons against the steep gradients. This dependency of cellular respiration on the ability to phosphorylate ADP to ATP is known as respiratory control and is the consequence of the tight coupling of these processes.

c. Uncoupling proteins: Uncoupling proteins (UCPs) occur in the inner mitochondrial membrane of mammals, including humans. These proteins form channels that allow protons to reenter the mitochondrial matrix without energy being captured as ATP (Figure 6.15). The energy is released as heat, and the process is called nonshivering thermogenesis. UCP1, also called thermogenin, is responsible for heat production in the brown adipocytes of mammals. In brown fat, unlike the more abundant white fat, almost 90% of its respiratory energy is used for thermogenesis in response to cold in the neonate and during arousal in hibernating animals. However, humans appear to have few concentrated deposits of brown fat (except in the newborn), and UCP1 does not appear to play a major role in energy balance. [Note: Uncoupling proteins UCP2–UCP5 have been found in other tissues, but their full significance remains unclear.]

d. **Synthetic uncouplers:** Electron transport and phosphorylation can also be uncoupled by compounds that pick up protons in the intermembrane space and release them in the matrix, dissipating the gradient. The classic example is 2,4-dinitrophenol, a lipophilic proton carrier that readily diffuses through the mitochondrial membrane. This uncoupler causes electron transport to proceed at a rapid rate without establishing a proton gradient, much as do the UCPs (see Figure 6.15). Again, energy is released as heat rather than being used to synthesize ATP. [Note: In high doses, aspirin and other salicylates uncouple oxidative phosphorylation. This explains the fever that accompanies toxic overdoses of these drugs.]



Uncoupling proteins create a channel, allowing protons (H^{+1}) to reenter the mitochondrial matrix without capturing any energy as ATP.

Figure 6.15
Transport of protons across the mitochondrial membrane by an uncoupling protein. ADP = adenosine diphosphate; ATP = adenosine triphosphate.

B. Membrane transport systems

The inner mitochondrial membrane is impermeable to most charged or hydrophilic substances. However, it contains numerous transport proteins that permit passage of specific molecules from the cytosol (or more correctly, the intermembrane space) to the mitochondrial matrix.

1. **ATP and ADP transport:** The inner membrane requires specialized carriers to transport ADP and P_i from the cytosol (where ATP is hydrolyzed to ADP in many energy-requiring reactions) into mitochondria, where ATP can be resynthesized. An adenine nucleotide antiporter imports one ADP from the cytosol into the matrix, while exporting one ATP from the matrix into the cytosol (see Figure 6.13). A transporter moves P_i from the cytosol into mitochondria.

2. **Transport of reducing equivalents:** The inner mitochondrial membrane lacks an NADH transporter, and NADH produced in the cytosol (for example, in glycolysis; see p. 101) cannot directly enter the mitochondrial matrix. However, two electrons (reducing equivalents) of NADH are transported from the cytosol into the matrix using substrate shuttles. In the glycerophosphate shuttle (Figure 6.16A), two electrons are transferred from NADH to dihydroxyacetone phosphate by cytosolic glycerophosphate dehydrogenase. The glycerol 3-phosphate produced is oxidized by the mitochondrial isozyme as FAD is reduced to $FADH_2$. CoQ of the ETC oxidizes the $FADH_2$. The glycerophosphate shuttle, therefore, results in the synthesis of two ATPs for each cytosolic NADH oxidized. This contrasts with the malate-aspartate shuttle (Figure 6.16B), which produces NADH (rather than $FADH_2$) in the mitochondrial matrix and, therefore, yields three ATPs for each cytosolic NADH oxidized by malate dehydrogenase as oxaloacetate is reduced to malate. A transport protein moves malate into the matrix.

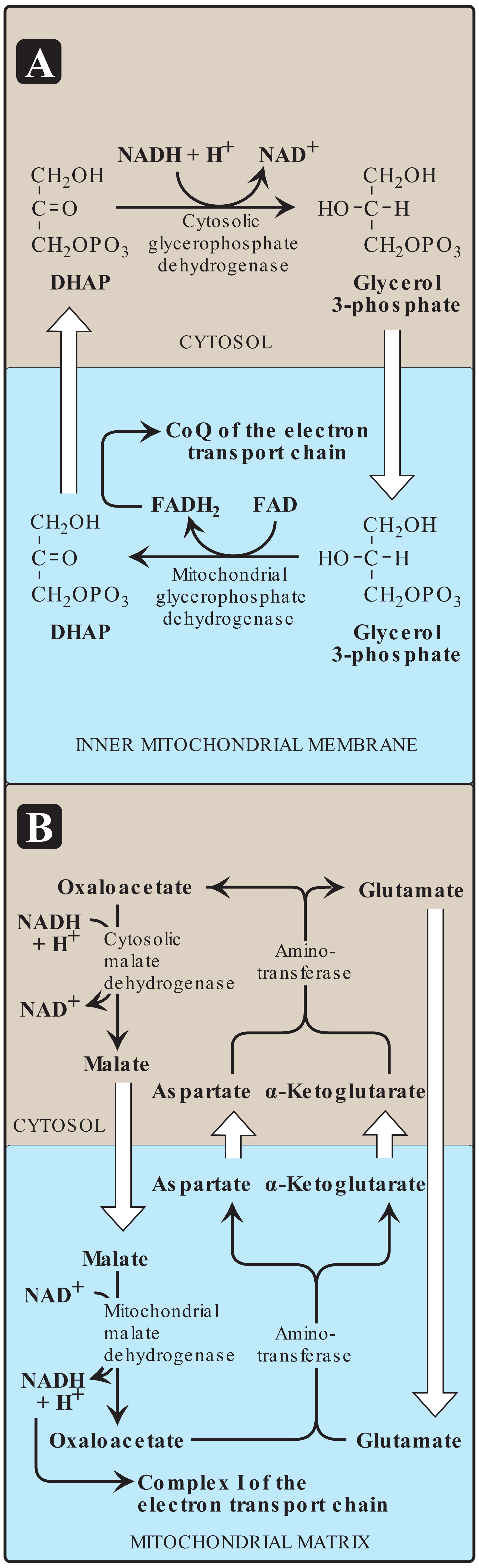

A
NADH + H+
NAD+
CH2OH
C=O
CH2OPO3
DHAP
Cytosolic glycerophosphate dehydrogenase
CH2OH
HO-C-H
CH2OPO3
Glycerol 3-phosphate
CYTOSOL
CoQ of the electron transport chain
FADH2
FAD
CH2OH
C=O
CH2OPO3
DHAP
Mitochondrial glycerophosphate dehydrogenase
CH2OH
HO-C-H
CH2OPO3
Glycerol 3-phosphate
INNER MITOCHONDRIAL MEMBRANE
B
Oxaloacetate
Glutamate
NADH + H+
Cytosolic malate dehydrogenase
NAD+
Amino-transferase
Malate
Aspartate
α-Ketoglutarate
CYTOSOL
Aspartate
α-Ketoglutarate
Malate
NAD+
Mitochondrial malate dehydrogenase
Amino-transferase
NADH + H+
Oxaloacetate
Glutamate
Complex I of the electron transport chain
MITOCHONDRIAL MATRIX

Figure 6.16
Substrate shuttles for the transport of electrons across the inner mitochondrial membrane.
A. Glycerophosphate shuttle.
B. Malate-aspartate shuttle.
DHAP = dihydroxyacetone phosphate; NAD(H) = nicotinamide adenine dinucleotide; FAD(H_2) = flavin adenine dinucleotide; CoQ = coenzyme Q.

C. Inherited defects in oxidative phosphorylation

Thirteen of the approximately 90 polypeptides required for oxidative phosphorylation are coded for by mtDNA and synthesized in mitochondria, whereas the remaining proteins are coded for by nuclear DNA, synthesized in the cytosol, and transported into mitochondria posttranslationally. Defects in oxidative phosphorylation are more likely a result of alterations in mtDNA, which has a mutation rate about 10 times greater than that of nuclear DNA. Tissues with the greatest ATP requirement (for example, central nervous system, skeletal and heart muscle, and liver) are most affected by defects in oxidative phosphorylation. Mutations in mtDNA are responsible for several diseases, including some cases of mitochondrial myopathies, and Leber hereditary optic neuropathy, a disease in which bilateral loss of central vision occurs as a result of neuroretinal degeneration, including damage to the optic nerve. [Note: mtDNA is maternally inherited because mitochondria from the sperm cell do not enter the fertilized egg.]

D. Mitochondria and apoptosis

The process of apoptosis, or programmed cell death, may be initiated through the intrinsic (mitochondrial-mediated) pathway by the formation of pores in the outer mitochondrial membrane. These pores allow cytochrome c to leave the intermembrane space and enter the cytosol. There, cytochrome c, in association with pro-apoptotic factors, activates a family of proteolytic enzymes (the caspases), causing cleavage of key proteins and resulting in the morphologic and biochemical changes characteristic of apoptosis.

VII. CHAPTER SUMMARY

The change in **free energy** (ΔG) occurring during a reaction predicts the direction in which that reaction will spontaneously proceed. If ΔG is **negative** (that is, the product has a lower free energy than the substrate), the **reaction goes spontaneously**. If ΔG is **positive**, the reaction **does not go spontaneously**. If $\Delta G = 0$, the reactions are in **equilibrium**. The ΔG of the forward reaction is equal in magnitude but opposite in sign to that of the back reaction. The ΔGs are **additive** in any sequence of consecutive reactions, as are the standard free energy changes (ΔG^os). Therefore, reactions or processes that have a large, positive ΔG are made possible by **coupling** with those that have a large, negative ΔG such as hydrolysis of **adenosine triphosphate (ATP)**. The reduced coenzymes **nicotinamide adenine dinucleotide (NADH)** and **flavin adenine dinucleotide ($FADH_2$)** each donate a pair of electrons to a specialized set of electron carriers, consisting of **flavin mononucleotide** (**FMN**), **iron-sulfur centers**, **coenzyme Q**, and a series of **cytochromes**, collectively called the **electron transport chain**. This pathway is present in the **inner mitochondrial membrane** (impermeable to most substances) and is the final common pathway by which electrons derived from different fuels of the body flow to O_2, reducing it to water. The terminal cytochrome, cytochrome oxidase, is the only cytochrome able to bind O_2. **Electron transport** results in the **pumping of protons** across the inner mitochondrial membrane from

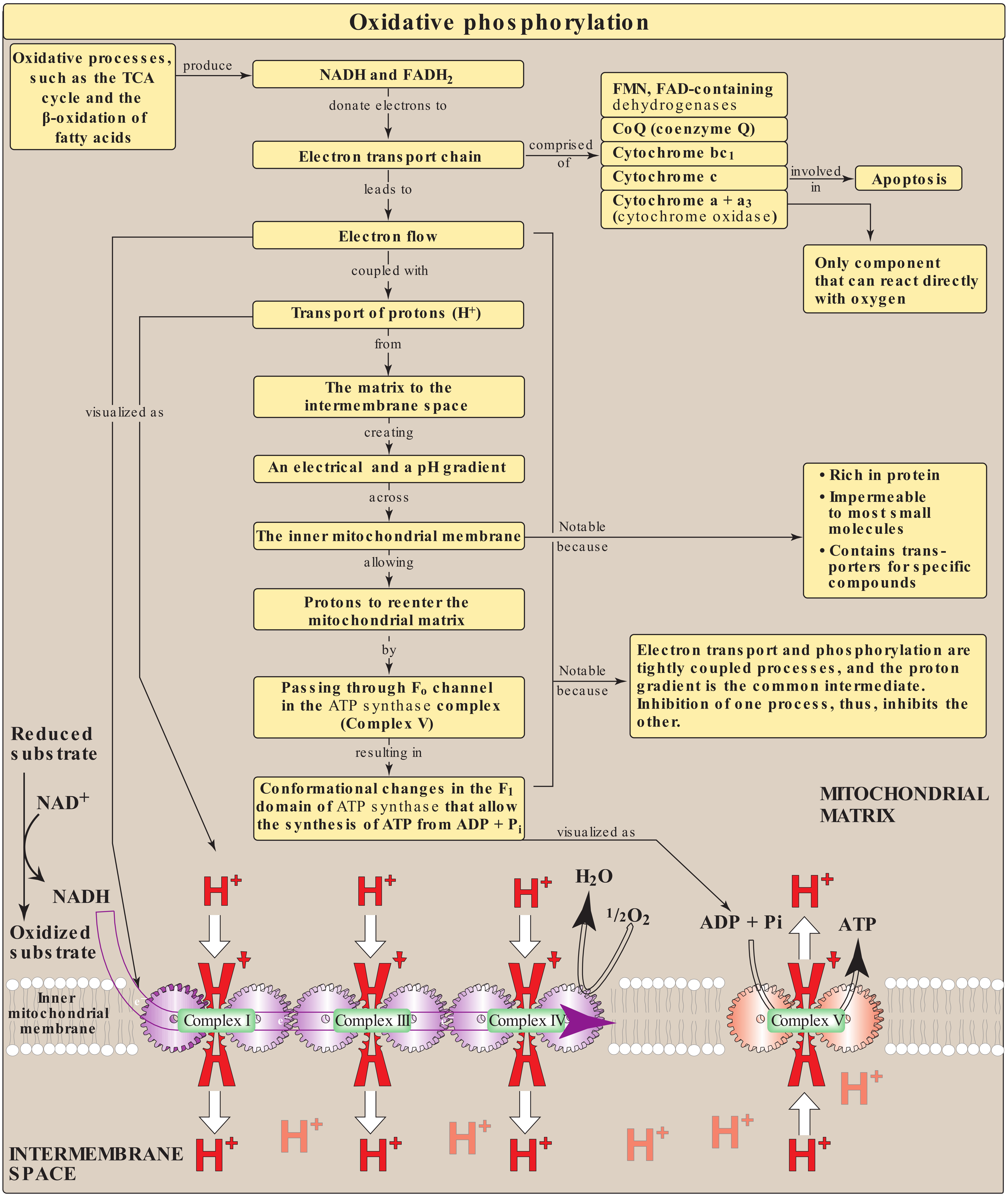

Oxidative phosphorylation
Oxidative processes, such as the TCA cycle and the β-oxidation of fatty acids
produce
NADH and FADH₂
donate electrons to
Electron transport chain
comprised of
FMN, FAD-containing dehydrogenases
CoQ (coenzyme Q)
Cytochrome bc₁
Cytochrome c
involved in
Apoptosis
Cytochrome a + a₃ (cytochrome oxidase)
Only component that can react directly with oxygen
leads to
Electron flow
coupled with
Transport of protons (H⁺)
from
The matrix to the intermembrane space
creating
An electrical and a pH gradient
across
The inner mitochondrial membrane
Notable because
• Rich in protein
• Impermeable to most small molecules
• Contains transporters for specific compounds
allowing
Protons to reenter the mitochondrial matrix
by
Passing through F₀ channel in the ATP synthase complex (Complex V)
resulting in
Conformational changes in the F₁ domain of ATP synthase that allow the synthesis of ATP from ADP + Pᵢ
Notable because
Electron transport and phosphorylation are tightly coupled processes, and the proton gradient is the common intermediate. Inhibition of one process, thus, inhibits the other.
visualized as
visualized as
Reduced substrate
NAD⁺
NADH
Oxidized substrate
MITOCHONDRIAL MATRIX
H⁺
H₂O
1/2O₂
ADP + Pi
ATP
Inner mitochondrial membrane
Complex I
Complex III
Complex IV
Complex V
e⁻
INTERMEMBRANE SPACE

Figure 6.17

Key concept map for oxidative phosphorylation (OXPHOS). [Note: Electron (e^-) flow and ATP synthesis are envisioned as sets of interlocking gears to emphasize the idea of coupling.] TCA = tricarboxylic acid; NAD(H) = nicotinamide adenine dinucleotide; FAD(H_2) = flavin adenine dinucleotide; FMN = flavin mononucleotide.

the matrix to the intermembrane space. This process creates **electrical** and **pH gradients** across the inner mitochondrial membrane. After protons have been transferred to the cytosolic side of the membrane, they reenter the matrix by passing through the F_o proton channel in ATP synthase (**Complex V**), dissipating the pH and electrical gradients and causing conformational changes in the β subunits of F_1 that result in the synthesis of ATP from adenosine diphosphate + inorganic phosphate. **Electron transport** and **phosphorylation** are **tightly coupled** in oxidative phosphorylation (OXPHOS, Figure 6.17). Inhibition of one process inhibits the other. These processes can be **uncoupled** by **uncoupling protein-1** of the inner mitochondrial membrane of cells in brown fat and by synthetic compounds such as **2,4-dinitrophenol** and **aspirin**, all of which dissipate the proton gradient. In uncoupled mitochondria, the energy produced by the transport of electrons is released as **heat** rather than being used to synthesize ATP. Mutations in **mitochondrial DNA**, which is maternally inherited, are responsible for some cases of **mitochondrial diseases** such as **Leber hereditary optic neuropathy**. The release of cytochrome c into the cytoplasm and subsequent activation of proteolytic caspases results in apoptotic cell death.

Introduction to Carbohydrates

7

I. OVERVIEW

Carbohydrates (saccharides) are the most abundant organic molecules in nature. They have a wide range of functions, including providing a significant fraction of the dietary calories for most organisms, acting as a storage form of energy in the body, and serving as cell membrane components that mediate some forms of intercellular communication. Carbohydrates also serve as a structural component of many organisms, including the cell walls of bacteria, the exoskeleton of many insects, and the fibrous cellulose of plants. The empiric formula for many of the simpler carbohydrates is $(CH_2O)_n$, where $n \geq 3$, hence the name "hydrate of carbon."

II. CLASSIFICATION AND STRUCTURE

Monosaccharides (simple sugars) can be classified according to the number of carbon atoms they contain. Examples of some monosaccharides commonly found in humans are listed in Figure 7.1. They can also be classified by the type of carbonyl group they contain. Carbohydrates with an aldehyde as their carbonyl group are called aldoses, whereas those with a keto as their carbonyl group are called ketoses (Figure 7.2). For example, glyceraldehyde is an aldose, whereas dihydroxyacetone is a ketose. Carbohydrates that have a free carbonyl group have the suffix –ose. [Note: Ketoses have an additional "ul" in their suffix such as xylulose. There are exceptions, such as fructose, to this rule.] Monosaccharides can be linked by glycosidic bonds to create larger structures (Figure 7.3). Disaccharides contain two monosaccharide units, oligosaccharides contain three to ten monosaccharide units, and polysaccharides contain more than ten monosaccharide units and can be hundreds of sugar units in length.

Generic names	Examples
3 Carbons: trioses	Glyceraldehyde
4 Carbons: tetroses	Erythrose
5 Carbons: pentoses	Ribose
6 Carbons: hexoses	Glucose
7 Carbons: heptoses	Sedoheptulose
9 Carbons: nonoses	Neuraminic acid

Figure 7.1
Examples of monosaccharides found in humans, classified according to the number of carbons they contain.

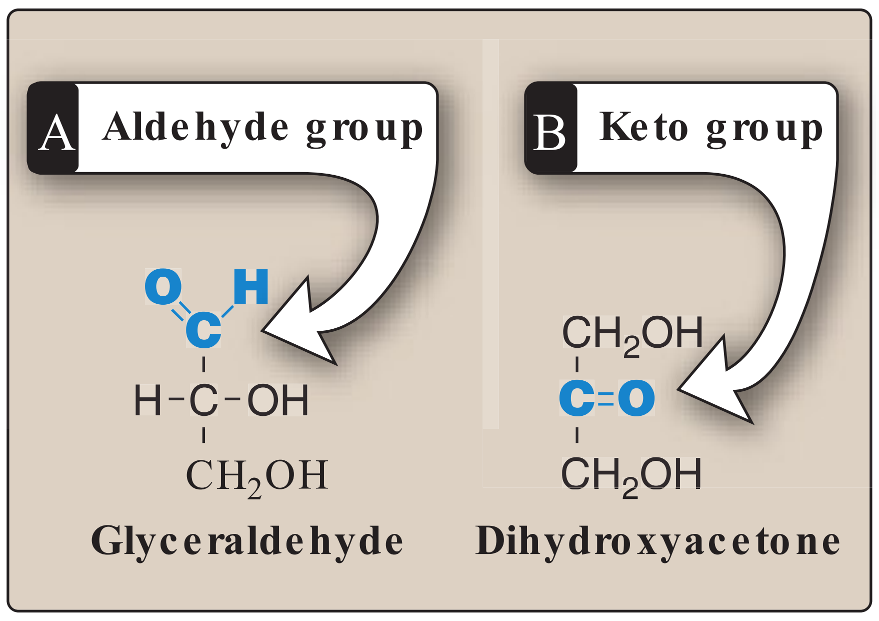


Figure 7.2
Examples of an aldose (A) and a ketose (B) sugar.

A. Isomers and epimers

Compounds that have the same chemical formula but have different structures are called isomers. For example, fructose, glucose, mannose, and galactose are all isomers of each other, having the same chemical formula, $C_6H_{12}O_6$. Carbohydrate isomers that differ in configuration around only one specific carbon atom (with the exception of the carbonyl carbon; see "anomers" below) are defined as epimers of each other. For example, glucose and galactose are

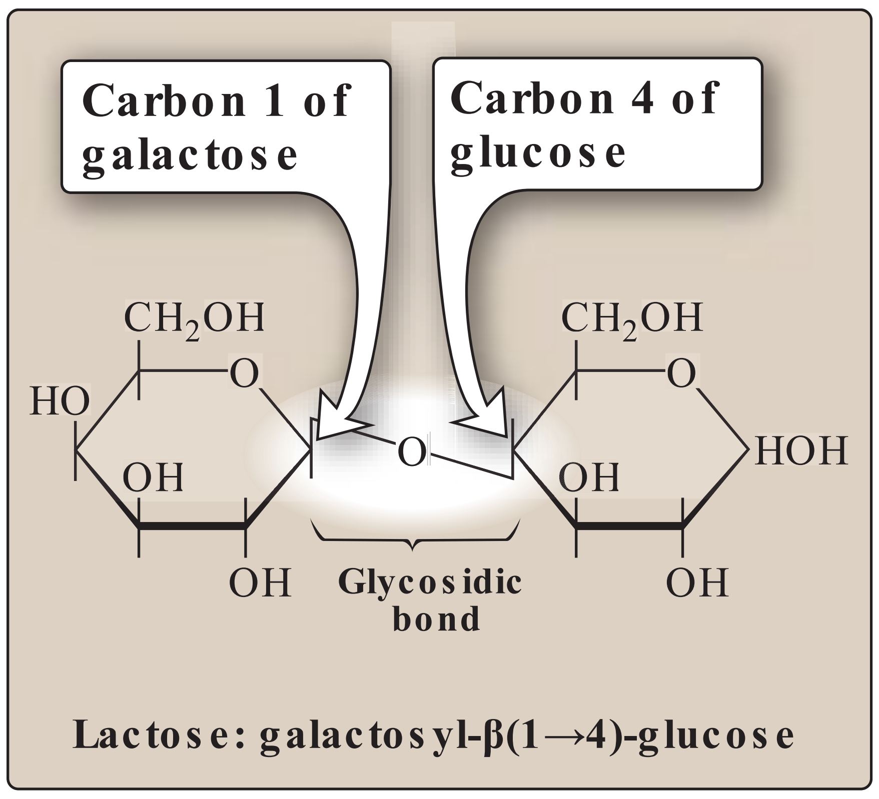


Figure 7.3
A glycosidic bond between two hexoses producing a disaccharide.

C-4 epimers because their structures differ only in the position of the –OH group at carbon 4. [Note: The carbons in sugars are numbered beginning at the end that contains the carbonyl carbon (that is, the aldehyde or keto group) as shown in Figure 7.4.] Glucose and mannose are C-2 epimers. However, because galactose and mannose differ in the position of –OH groups at two carbons (carbons 2 and 4), they are isomers rather than epimers (see Figure 7.4).

B. Enantiomers

A special type of isomerism is found in the pairs of structures that are mirror images of each other. These mirror images are called enantiomers, and the two members of the pair are designated as a D- and an L-sugar (Figure 7.5). The vast majority of the sugars in humans are D-sugars. In the D isomeric form, the –OH group on the asymmetric carbon (a carbon linked to four different atoms or groups) farthest from the carbonyl carbon is on the right, whereas in the L-isomer, it is on the left. Most enzymes are specific for either the D or the L form, but enzymes known as racemases are able to interconvert D- and L-isomers.

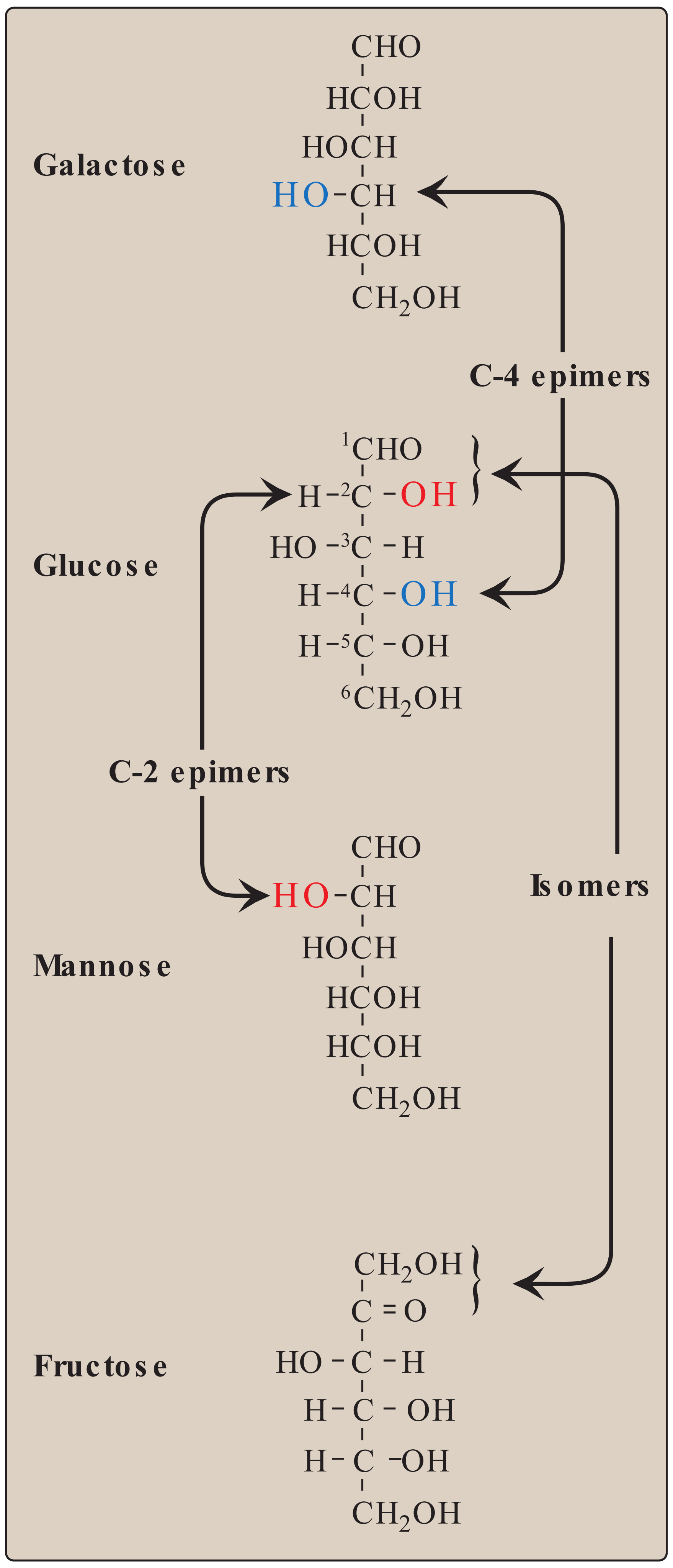


Figure 7.4
C-2 and C-4 epimers and an isomer of glucose.

C. Cyclization of monosaccharides

Less than 1% of each of the monosaccharides with five or more carbons exists in the open-chain (acyclic) form in solution. Rather, they are predominantly found in a ring (cyclic) form, in which the aldehyde (or keto) group has reacted with an alcohol group on the same sugar, making the carbonyl carbon (carbon 1 for an aldose, carbon 2 for a ketose) asymmetric. This asymmetric carbon is referred to as the anomeric carbon.

1. **Anomers:** Creation of an anomeric carbon (the former carbonyl carbon), generates a new pair of isomers, the α and β configurations of the sugar (for example, α-D-glucopyranose and β-D-glucopyranose; see Figure 7.6), that are anomers of each other. [Note: In the α configuration, the –OH group on the anomeric carbon projects to the same side as the ring in a modified Fischer projection formula (Figure 7.6A) and is trans to the CH_2OH group in a Haworth projection formula (Figure 7.6B). The α and β forms are not mirror images, and they are referred to as diastereomers.] Enzymes are able to distinguish between these two structures and use one or the other preferentially. For example, glycogen is synthesized from α-D-glucopyranose, whereas cellulose is synthesized from β-D-glucopyranose. The cyclic α and β anomers of a sugar in solution spontaneously (but slowly) form an equilibrium mixture, a process known as mutarotation (see Figure 7.6). [Note: For glucose, the α form makes up 36% of the mixture.]

2. **Reducing sugars:** If the hydroxyl group on the anomeric carbon of a cyclized sugar is not linked to another compound by a glycosidic bond, the ring can open. The sugar can act as a reducing agent and is termed a reducing sugar. Such sugars can react with chromogenic agents (for example, the Benedict reagent) causing the reagent to be reduced and colored, with the aldehyde group of the acyclic sugar becoming oxidized. All monosaccharides, but not all disaccharides, are reducing sugars. [Note:

Glucose can have its terminal hydroxyl group oxidized to a carboxyl group, forming glucuronic acid (see p. 161), or its aldehyde group oxidized to a hydroxyl group, forming a sugar alcohol.]

A colorimetric test can detect a reducing sugar in urine. A positive result is indicative of an underlying pathology, because sugars are not normally present in urine, and can be followed up by more specific tests to identify the reducing sugar.

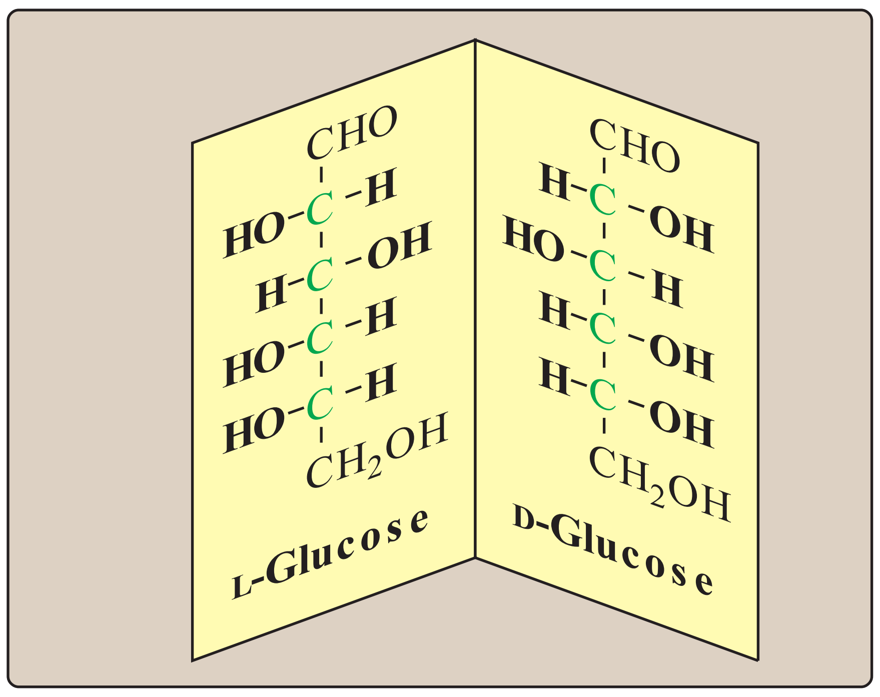

CHO
HO-C-H
H-C-OH
HO-C-H
HO-C-H
CH₂OH
L-Glucose
CHO
H-C-OH
HO-C-H
H-C-OH
H-C-OH
CH₂OH
D-Glucose

Figure 7.5
Enantiomers (mirror images) of glucose. Designation of D and L is by comparison to the triose, glyceraldehyde. [Note: The asymmetric carbons are shown in green.]

D. Joining of monosaccharides

Monosaccharides can be joined to form disaccharides, oligosaccharides, and polysaccharides. Important disaccharides include lactose (galactose + glucose), sucrose (glucose + fructose), and maltose (glucose + glucose). Important polysaccharides include branched glycogen (from animal sources) and starch (plant sources) and unbranched cellulose (plant sources). Each is a polymer of glucose. The bonds that link sugars are called glycosidic bonds. These are formed by enzymes known as glycosyltransferases that use nucleotide sugars such as uridine diphosphate glucose as substrates.

1. **Naming glycosidic bonds:** Glycosidic bonds between sugars are named according to the numbers of the connected carbons and with regard to the position of the anomeric hydroxyl group of the sugar involved in the bond. If this anomeric hydroxyl is in the α configuration, the linkage is an α-bond. If it is in the β configuration, the linkage is a β-bond. Lactose, for example, is synthesized by forming a glycosidic bond between carbon 1 of β-galactose and carbon 4 of glucose. The linkage is, therefore, a β(1→4) glycosidic bond (see Figure 7.3). [Note: Because the anomeric end of the glucose residue is not involved in the glycosidic linkage, it (and, therefore, lactose) remains a reducing sugar.]

E. Complex carbohydrates

Carbohydrates can be attached by glycosidic bonds to noncarbohydrate structures, including purine and pyrimidine bases (found in

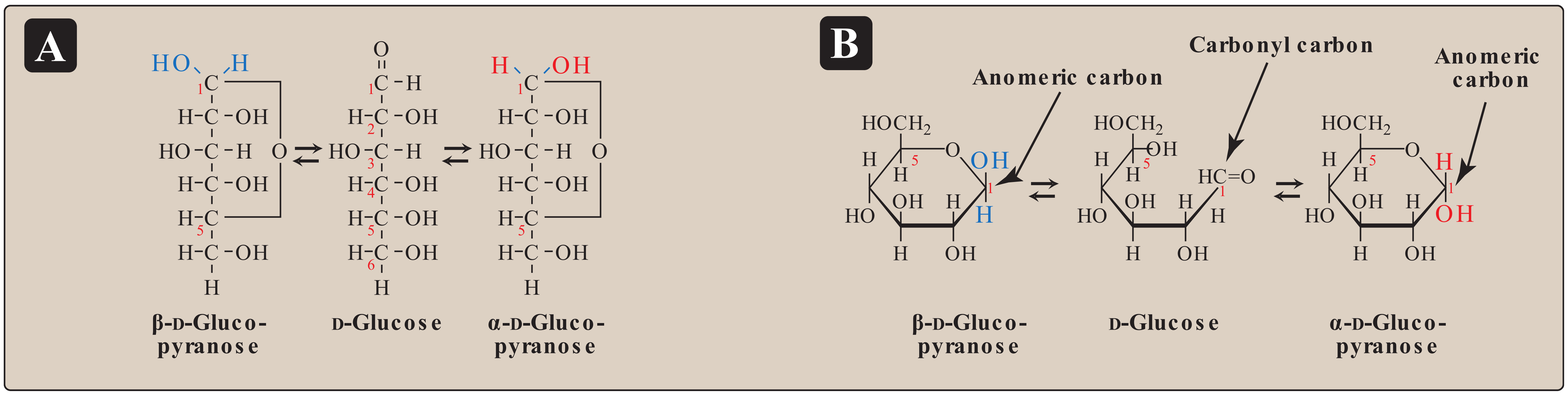

A
HO H
H-C-OH
HO-C-H
H-C-OH
H-C
H-C-OH
H
O
C-H
H-C-OH
HO-C-H
H-C-OH
H-C-OH
H-C-OH
H
H OH
β-D-Glucopyranose
D-Glucose
α-D-Glucopyranose
B
Anomeric carbon
Carbonyl carbon
Anomeric carbon
HOCH₂
OH
HC=O
H
OH
HO
β-D-Glucopyranose
D-Glucose
α-D-Glucopyranose

Figure 7.6
A The interconversion (mutarotation) of the α and β anomeric forms of glucose shown as modified Fischer projection formulas. B. The interconversion shown as Haworth projection formulas. [Note: A sugar with a six-membered ring (5C + 1O) is termed a pyranose, whereas one with a five-membered ring (4C + 1O) is a furanose. Virtually all glucose in solution is in the pyranose form.]

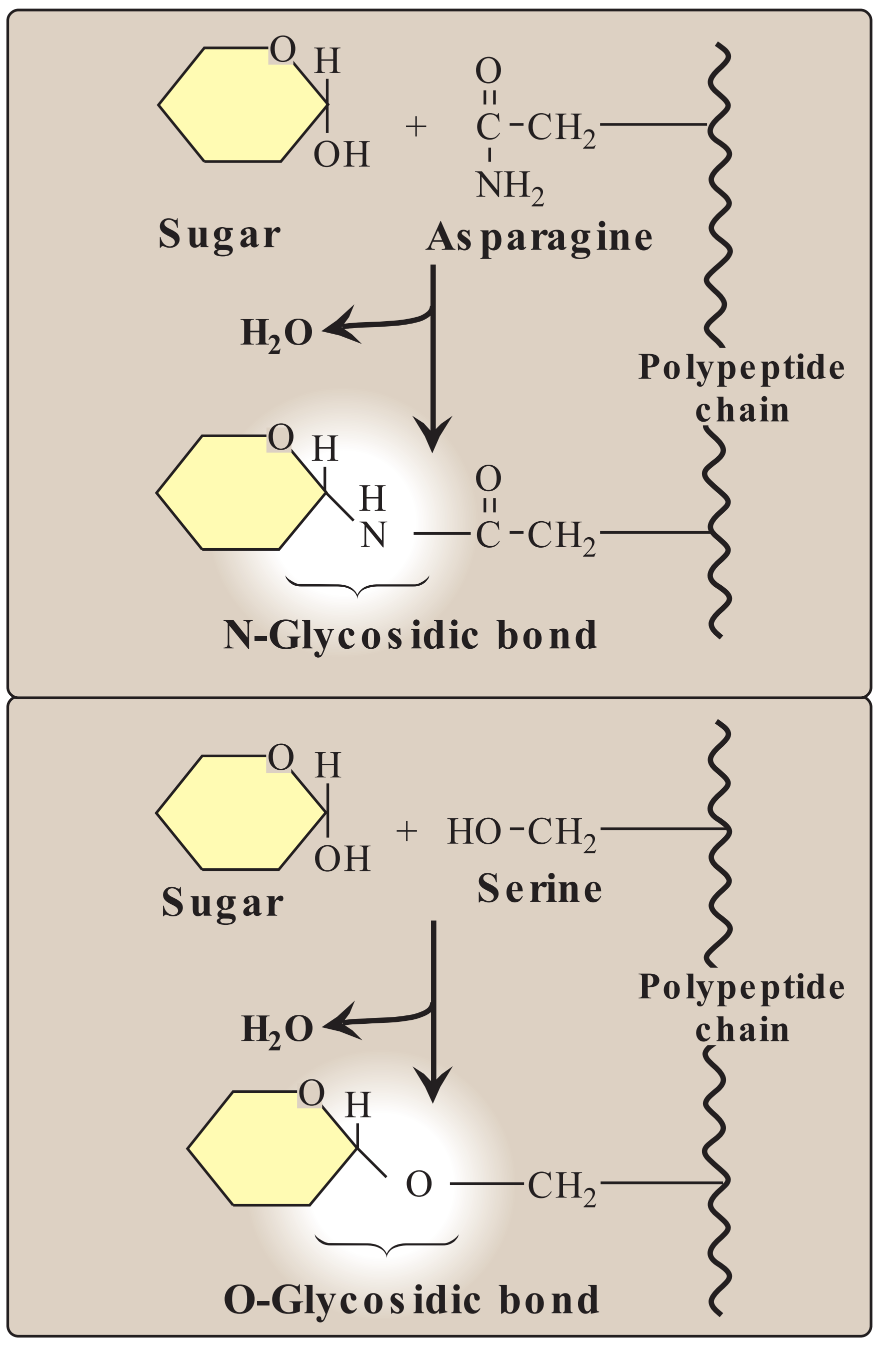


Figure 7.7
Glycosides: examples of N- and O-glycosidic bonds.

nucleic acids), aromatic rings (such as those found in steroids and bilirubin), proteins (found in glycoproteins and proteoglycans), and lipids (found in glycolipids), to form glycosides.

1. **N- and O-glycosides:** If the group on the noncarbohydrate molecule to which the sugar is attached is an $-NH_2$ group, the structure is an N-glycoside, and the bond is called an N-glycosidic link. If the group is an –OH, the structure is an O-glycoside, and the bond is an O-glycosidic link (Figure 7.7). [Note: All sugar–sugar glycosidic bonds are O-type linkages.]

III. DIGESTION OF DIETARY CARBOHYDRATES

The principal sites of dietary carbohydrate digestion are the mouth and intestinal lumen. This digestion is rapid and is catalyzed by enzymes known as glycoside hydrolases (glycosidases) that hydrolyze glycosidic bonds (Figure 7.8). Because there is little monosaccharide present in diets of mixed animal and plant origin, the enzymes are primarily endoglycosidases that hydrolyze polysaccharides and oliosaccharides, and disaccharidases that hydrolyse tri- and disaccharides into their reducing sugar components. Glycosidases are usually specific for the structure and configuration of the glycosyl residue to be removed as well as for the type of bond to be broken. The final products of carbohydrate digestion are the monosaccharides, glucose, galactose, and fructose that are absorbed by cells of the small intestine.

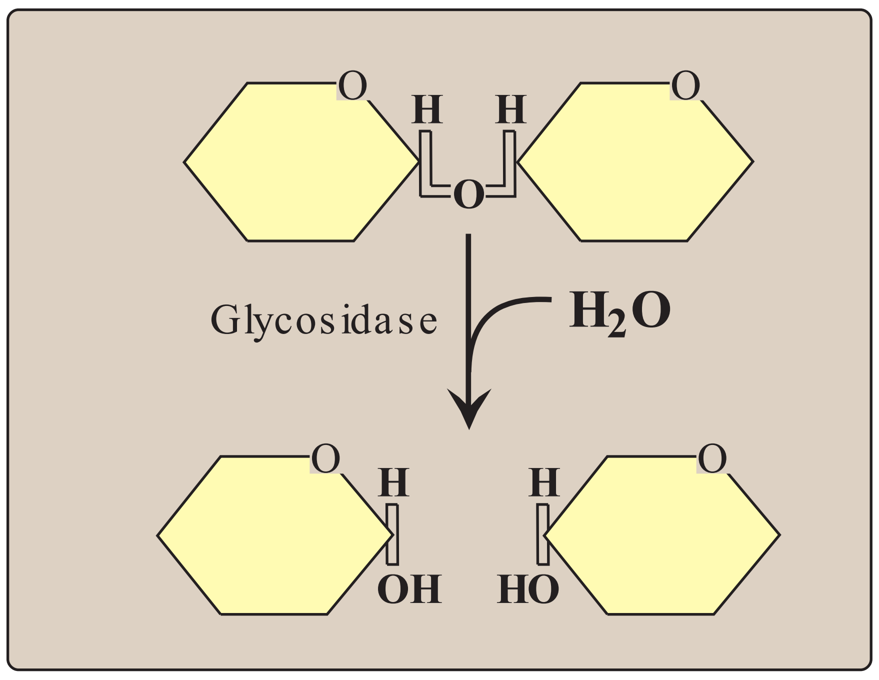


Figure 7.8
Hydrolysis of a glycosidic bond.

A. Salivary a-amylase

The major dietary polysaccharides are of plant (starch, composed of amylose and amylopectin) and animal (glycogen) origin. During mastication, salivary *α*-amylase acts briefly on dietary starch and glycogen, hydrolyzing random α(1→4) bonds. [Note: There are both *α*(1→4)- and *β*(1→4)-endoglucosidases in nature, but humans do not produce the latter. Therefore, we are unable to digest cellulose, a carbohydrate of plant origin containing β(1→4) glycosidic bonds between glucose residues.] Because branched amylopectin and glycogen also contain α(1→6) bonds, which *α*-amylase cannot hydrolyze, the digest resulting from its action contains a mixture of short, branched and unbranched oligosaccharides known as dextrins (Figure 7.9). [Note: Disaccharides are also present as they, too, are resistant to amylase.] Carbohydrate digestion halts temporarily in the stomach, because the high acidity inactivates salivary *α*-amylase.

B. Pancreatic a-amylase

When the acidic stomach contents reach the small intestine, they are neutralized by bicarbonate secreted by the pancreas, and pancreatic *α*-amylase continues the process of starch digestion.

C. Intestinal disaccharidases

The final digestive processes occur primarily at the mucosal lining of the upper jejunum and include the action of several disaccharidases (see Figure 7.9). For example, isomaltase cleaves the α(1→6) bond in isomaltose, and maltase cleaves the $\alpha(1\rightarrow4)$ bond in maltose and maltotriose, each producing glucose. Sucrase cleaves the $\alpha(1\rightarrow2)$ bond in sucrose, producing glucose and fructose, and lactase (β-galactosidase) cleaves the $\beta(1\rightarrow4)$ bond in lactose, producing galactose and glucose. [Note: The substrates for isomaltase are broader than its name suggests, and it hydrolyzes the majority of maltose.] Trehalose, an $\alpha(1\rightarrow1)$ disaccharide of glucose found in mushrooms and other fungi is cleaved by trehalase. These enzymes are transmembrane proteins of the brush border on the luminal surface of the intestinal mucosal cells.

> Sucrase and isomaltase are enzymic activities of a single protein (SI) which is cleaved into two functional subunits that remain associated in the cell membrane, forming the sucrase-isomaltase complex. In contrast, maltase is one of two enzymic activities of a single membrane protein maltase-glucoamylase (MGA) that does not get cleaved. Its second enzymic activity, glucoamylase, cleaves $\alpha(1\rightarrow4)$ glycosidic bonds in dextrins.

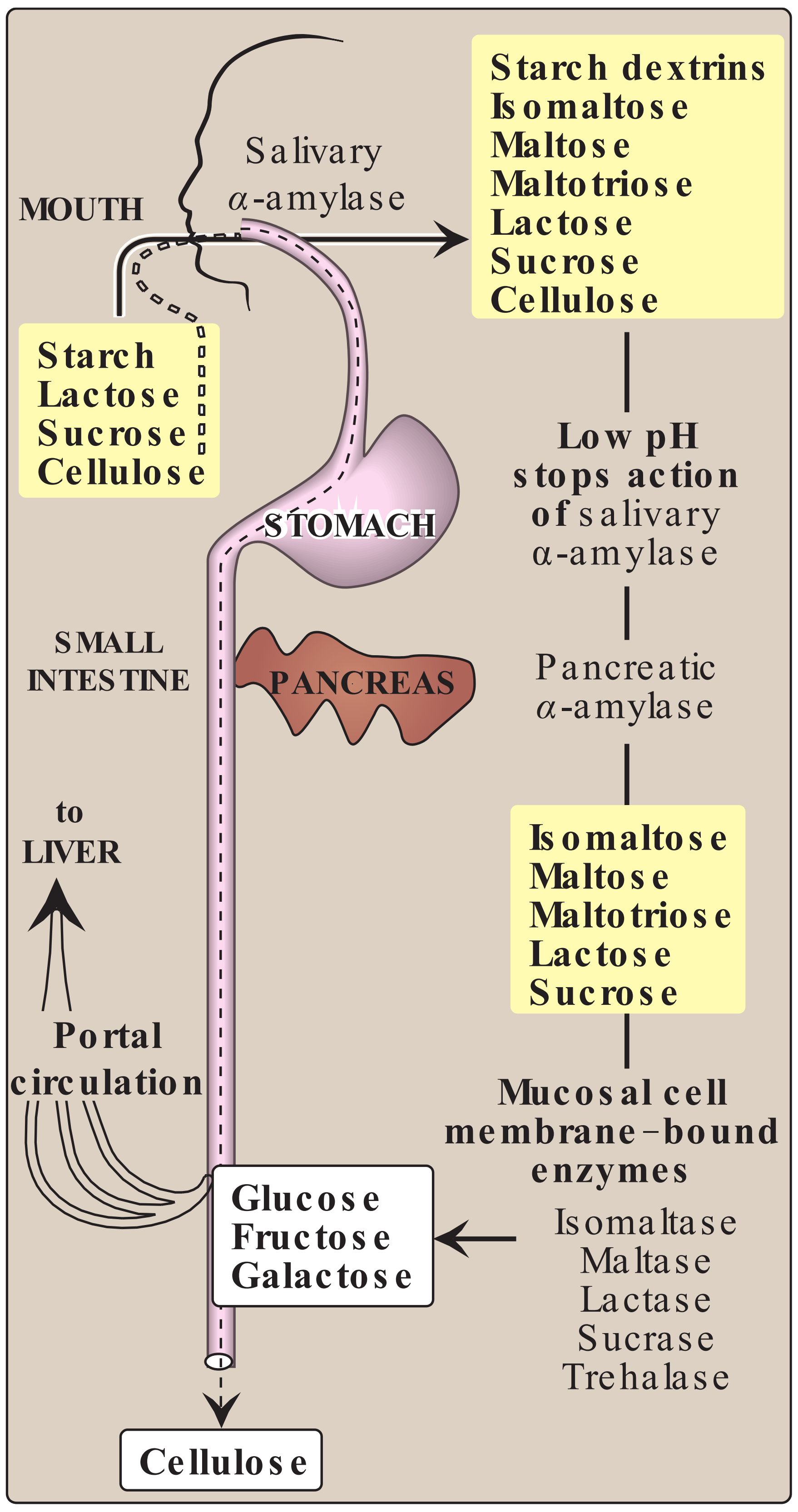

MOUTH
Salivary
α-amylase
Starch dextrins
Isomaltose
Maltose
Maltotriose
Lactose
Sucrose
Cellulose
Starch
Lactose
Sucrose
Cellulose
STOMACH
Low pH
stops action
of salivary
α-amylase
SMALL
INTESTINE
PANCREAS
Pancreatic
α-amylase
to
LIVER
Isomaltose
Maltose
Maltotriose
Lactose
Sucrose
Portal
circulation
Mucosal cell
membrane–bound
enzymes
Glucose
Fructose
Galactose
Isomaltase
Maltase
Lactase
Sucrase
Trehalase
Cellulose

Figure 7.9
Digestion of carbohydrates. [Note: Indigestible cellulose enters the colon and is excreted.]

D. Intestinal absorption of monosaccharides

The duodenum and upper jejunum absorb the bulk of the monosaccharide products of digestion. However, different sugars have different mechanisms of absorption (Figure 7.10). For example, galactose and glucose are transported into the mucosal cells by an active, energy-dependent process that requires a concurrent uptake of sodium ions, and the transport protein is the sodium-dependent glucose cotransporter 1 (SGLT-1). Fructose utilizes an energy- and sodium-independent monosaccharide transporter (GLUT-5) for its absorption. All three monosaccharides are transported from the intestinal mucosal cell into the portal circulation by yet another transporter, GLUT-2. (See p. 97 for a discussion of these transporters.)

E. Abnormal degradation of disaccharides

The overall process of carbohydrate digestion and absorption is so efficient in healthy individuals that ordinarily all digestible dietary carbohydrate is absorbed by the time the ingested material reaches the lower jejunum. However, because only monosaccharides are absorbed, any deficiency (genetic or acquired) in a specific disaccharidase activity of the intestinal mucosa causes the passage of undigested carbohydrate into the large intestine. As a consequence of the presence of this osmotically active material, water is drawn from the mucosa into the large intestine, causing osmotic diarrhea. This is reinforced by the bacterial fermentation of the remaining carbohydrate to two- and three-carbon compounds (which are also osmotically active) plus large volumes of CO_2 and H_2 gas, causing abdominal cramps, diarrhea, and flatulence.

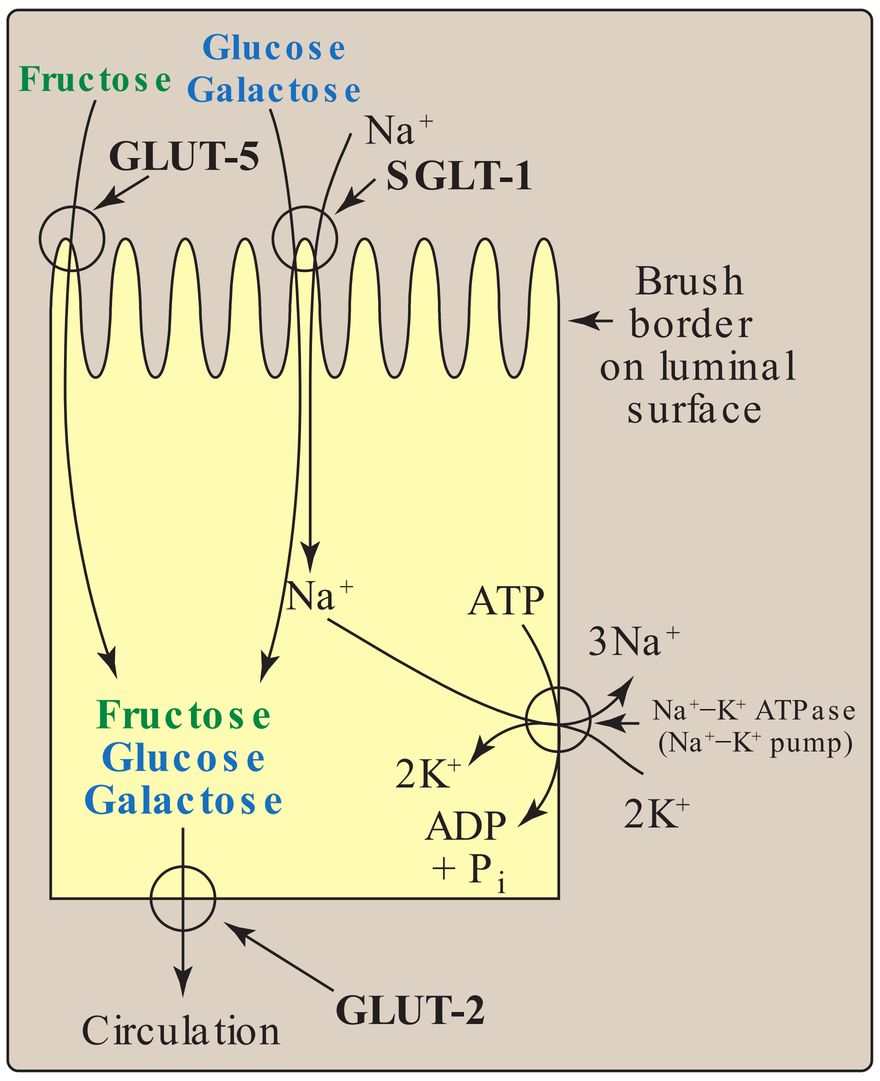

Fructose
Glucose
Galactose
Na^+
GLUT-5
SGLT-1
Brush
border
on luminal
surface
Na^+
ATP
$3Na^+$
Na^+–K^+ ATPase
(Na^+–K^+ pump)
Fructose
Glucose
Galactose
$2K^+$
ADP
+ P_i
$2K^+$
Circulation
GLUT-2

Figure 7.10
Absorption by intestinal mucosal cells of the monosaccharide products of carbohydrate digestion. SGLT-1 = sodium-dependent glucose transporter.

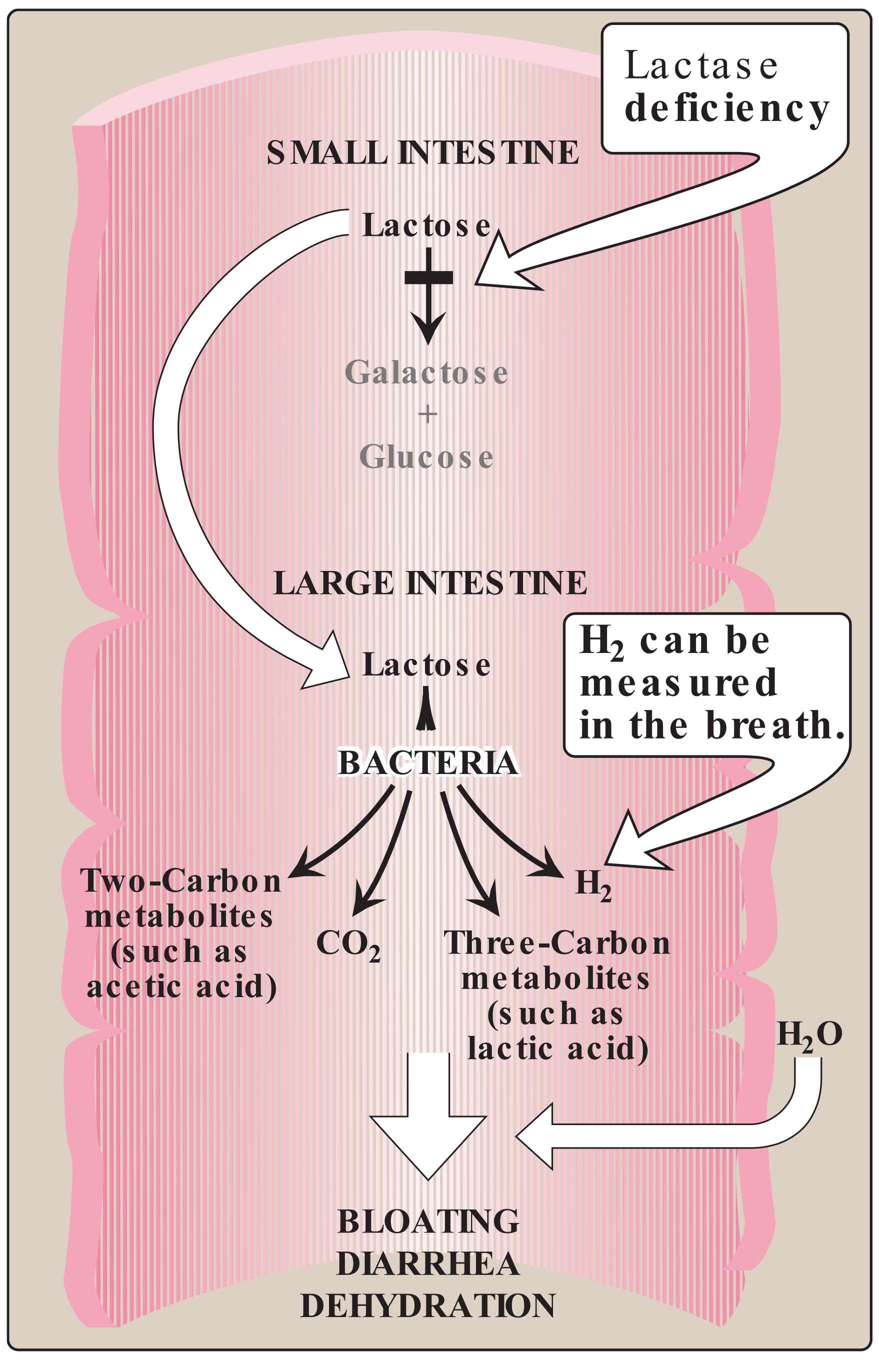


Figure 7.11
Abnormal lactose metabolism. H_2 = hydrogen gas.

1. **Digestive enzyme deficiencies:** Genetic deficiencies of the individual disaccharidases result in disaccharide intolerance. Alterations in disaccharide degradation can also be caused by a variety of intestinal diseases, malnutrition, and drugs that injure the mucosa of the small intestine. For example, brush border enzymes are rapidly lost in normal individuals with severe diarrhea, causing a temporary, acquired enzyme deficiency. Therefore, patients suffering or recovering from such a disorder cannot drink or eat significant amounts of dairy products or sucrose without exacerbating the diarrhea.

2. **Lactose intolerance:** More than 70% of the world's adults are-lactose intolerant (Figure 7.11). This is particularly manifested in certain populations. For example, up to 90% of adults of African or Asian descent are lactase-deficient and, therefore, are less able to metabolize lactose than individuals of Northern European origin. The age-dependent loss of lactase activity represents a reduction in the amount of enzyme produced. It is thought to be caused by small variations in the DNA sequence of a region on chromosome 2 that controls expression of the gene for lactase, also on chromosome 2. Treatment for this disorder is to reduce consumption of milk and eat yogurts and some cheeses (bacterial action and aging process decrease lactose content) as well as green vegetables, such as broccoli, to ensure adequate calcium intake; to use lactase-treated products; or to take lactase in pill form prior to eating. [Note: Because the loss of lactase is the norm for most of the world's adults, use of the term "adult hypolactasia" for lactose intolerance is becoming more common.] Rare cases of congenital lactase deficiency are known.

3. **Congenital sucrase-isomaltase deficiency:** This autosomal recessive disorder results in an intolerance of ingested sucrose. Congenital sucrase-isomaltase deficiency has a prevalence of 0.02% in individuals of European descent and appears to be much more common in the Inuit people of Greenland and Canada. Treatment includes the dietary restriction of sucrose and enzyme replacement therapy.

4. **Diagnosis:** Identification of a specific enzyme deficiency can be obtained by performing oral tolerance tests with the individual disaccharides. Measurement of hydrogen gas in the breath is a reliable test for determining the amount of ingested carbohydrate not absorbed by the body, but which is metabolized instead by the intestinal flora (see Figure 7.11).

IV. CHAPTER SUMMARY

Monosaccharides (Figure 7.12) containing an aldehyde group are called **aldoses**, and those with a keto group are called **ketoses**. **Disaccharides**, **oligosaccharides**, and **polysaccharides** consist of monosaccharides linked by **glycosidic bonds**. Compounds with the same chemical formula but different structures are called **isomers**. If two monosaccharide isomers differ in configuration around one specific carbon atom (with the exception of the carbonyl carbon), they are defined as **epimers** of each other. If a pair of sugars are mirror images (**enantiomers**), the two members of the pair are designated as **D-** and **L-sugars**. If the aldehyde group on an acyclic sugar gets oxidized as a chromogenic agent gets reduced, that sugar is a reducing sugar. When a sugar cyclizes, an **anomeric carbon** is created from the aldehyde group of an aldose or keto group of a ketose. The sugar can have two configurations, **a** or **β**. A sugar with its anomeric carbon linked to another structure forms a **glycoside**. Sugars can be attached either to an $-NH_2$ or an –OH group, producing **N-** and **O-glycosides**. Salivary α-amylase acts on **dietary polysaccharides** (starch, glycogen), producing oligosaccharides. Pancreatic α-amylase continues the process of carbohydrate digestion.The final digestive processes occur at the **mucosal lining** of the **small intestine**. Several disaccharidases (for example, lactase [β-galactosidase], sucrase, isomaltase, and maltase) produce monosaccharides (glucose, galactose, and fructose). These enzymes are **transmembrane proteins** of the luminal **brush border** of **intestinal mucosal cells**. Absorption of the monosaccharides requires specific transporters. If carbohydrate degradation is deficient (as a result of heredity, disease, or drugs that injure the intestinal mucosa), undigested carbohydrate will pass into the large intestine, where it can cause **osmotic diarrhea**. Bacterial fermentation of the material produces large volumes of CO_2 and H_2, causing abdominal cramps, diarrhea, and flatulence. **Lactose intolerance**, primarily caused by the age-dependent loss of lactase (**adult hypolactasia**), is by far the most common of these deficiencies.

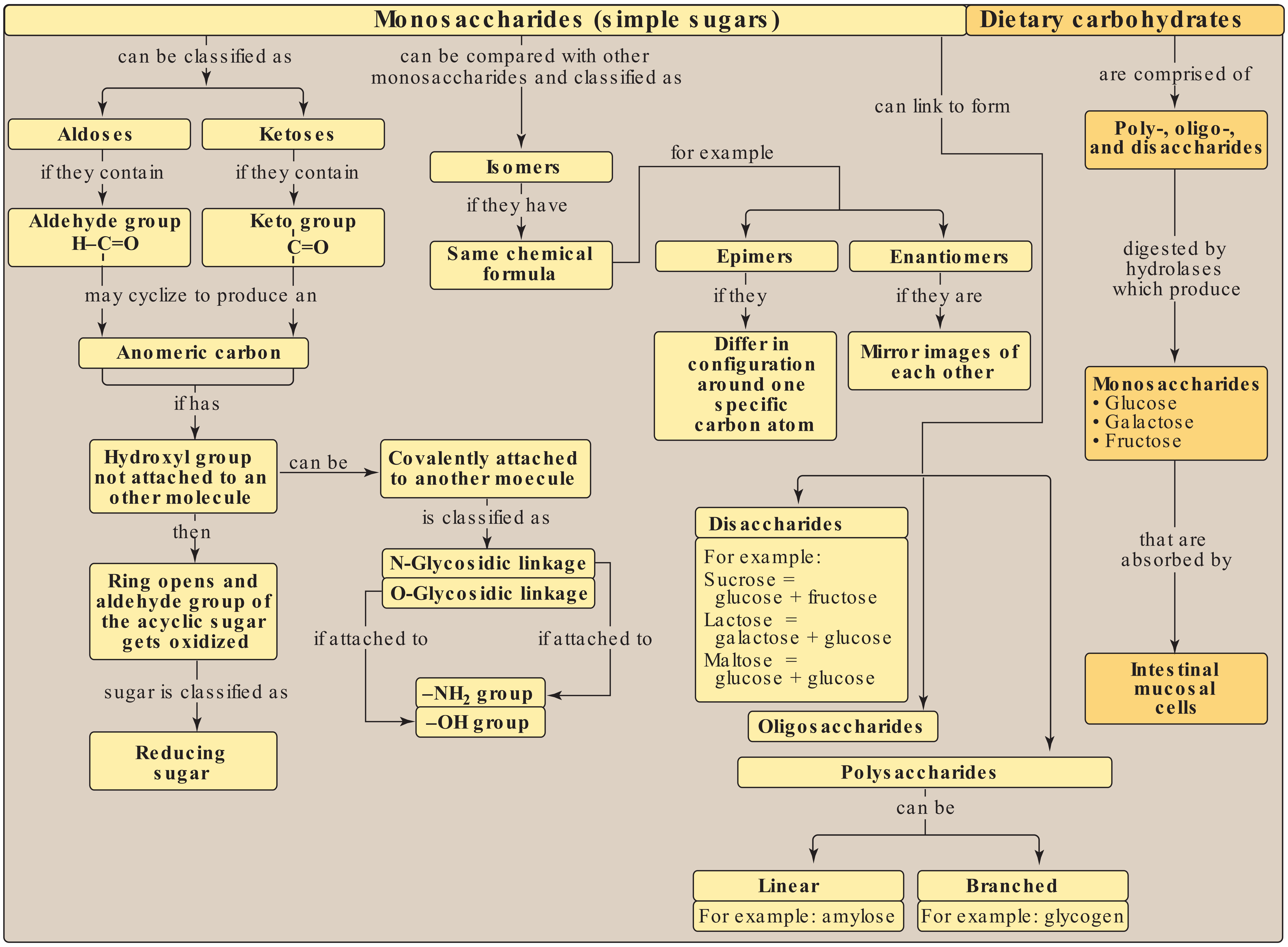

Monosaccharides (simple sugars)
Dietary carbohydrates
can be classified as
can be compared with other monosaccharides and classified as
can link to form
are comprised of
Aldoses
Ketoses
if they contain
if they contain
Aldehyde group H–C=O
Keto group C=O
Isomers
if they have
Same chemical formula
for example
Epimers
Enantiomers
if they
if they are
Differ in configuration around one specific carbon atom
Mirror images of each other
may cyclize to produce an
Anomeric carbon
if has
Hydroxyl group not attached to an other molecule
can be
Covalently attached to another moecule
is classified as
N-Glycosidic linkage
O-Glycosidic linkage
if attached to
if attached to
–NH₂ group
–OH group
then
Ring opens and aldehyde group of the acyclic sugar gets oxidized
sugar is classified as
Reducing sugar
Poly-, oligo-, and disaccharides
digested by hydrolases which produce
Monosaccharides
• Glucose
• Galactose
• Fructose
that are absorbed by
Intestinal mucosal cells
Disaccharides
For example:
Sucrose = glucose + fructose
Lactose = galactose + glucose
Maltose = glucose + glucose
Oligosaccharides
Polysaccharides
can be
Linear
For example: amylose
Branched
For example: glycogen

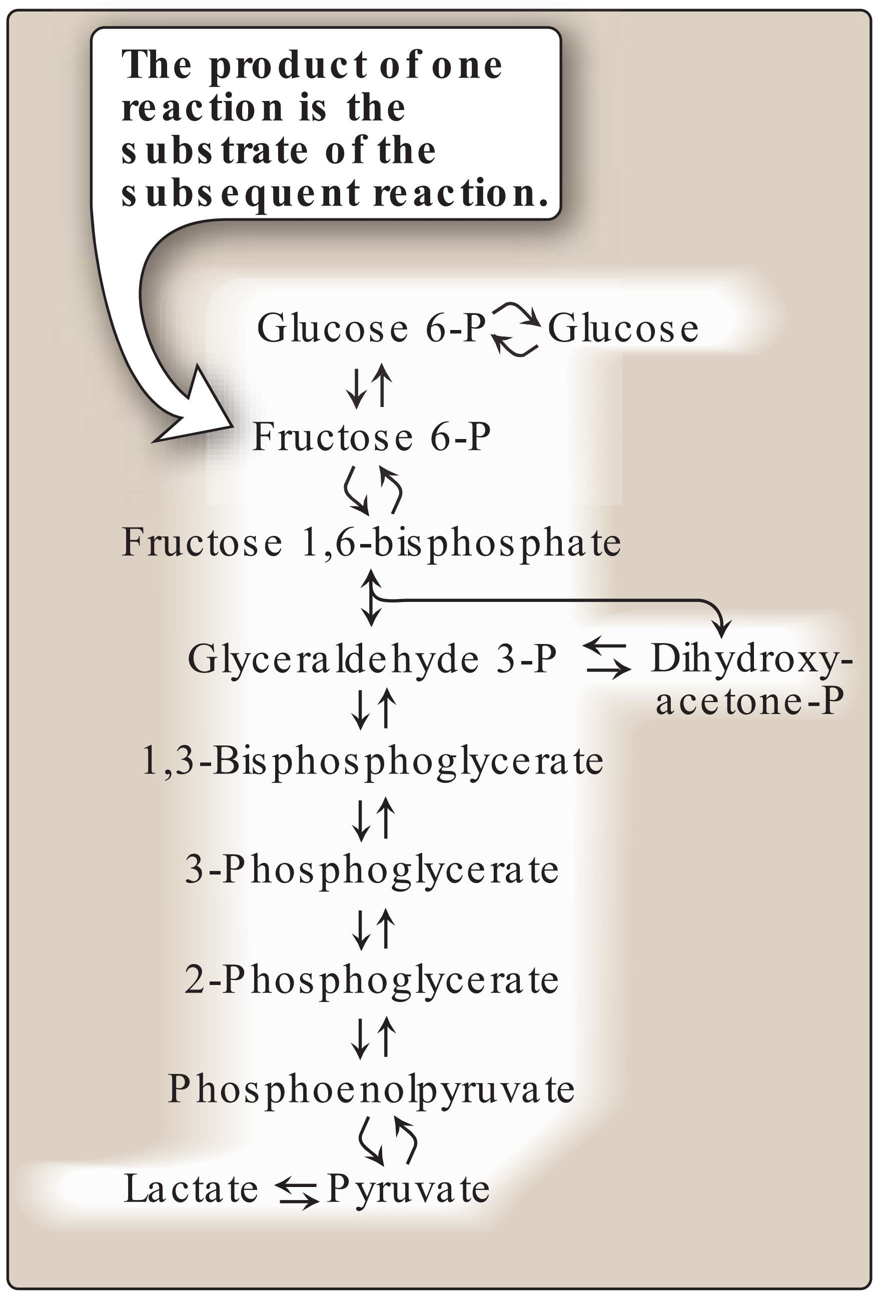
Figure 7.12
Key concept map for the classification and structure of monosaccharides and the digestion of dietary carbohydrates.

8

Introduction to Metabolism and Glycolysis

I. INTRODUCTION TO METABOLISM

In Chapter 5, individual enzymic reactions were analyzed in an effort to explain the mechanisms of catalysis. However, in cells, these reactions rarely occur in isolation but, rather, are organized into multistep sequences called pathways, such as that of glycolysis (Figure 8.1). In a pathway, the product of one reaction serves as the substrate of the subsequent reaction. Different pathways can also intersect, forming an integrated and purposeful network of chemical reactions. These are collectively called metabolism, which is the sum of all the chemical changes occurring in a cell, a tissue, or the body. Most pathways can be classified as either catabolic (degradative) or anabolic (synthetic). Catabolic reactions break down complex molecules, such as proteins, polysaccharides, and lipids, to a few simple molecules (for example, CO_2, NH_3 [ammonia], and H_2O). Anabolic pathways form complex end products from simple precursors, for example, the synthesis of the polysaccharide, glycogen, from glucose. [Note: Pathways that regenerate a component are called cycles.] In the following chapters, this text focuses on the central metabolic pathways that are involved in synthesizing and degrading carbohydrates, lipids, and amino acids.



The product of one reaction is the substrate of the subsequent reaction.

Figure 8.1
Glycolysis, an example of a metabolic pathway. [Note: Pyruvate to phosphoenolpyruvate requires two reactions.] Curved reaction arrows (⇄) indicate forward and reverse reactions that are catalyzed by different enzymes. P = phosphate.

A. Metabolic map

It is convenient to investigate metabolism by examining its component pathways. Each pathway is composed of multienzyme sequences, and each enzyme, in turn, may exhibit important catalytic or regulatory features. To provide the reader with the "big picture," a metabolic map containing the important central pathways of energy metabolism is presented in Figure 8.2. This map is useful in tracing connections between pathways, visualizing the purposeful "movement" of metabolic intermediates, and depicting the effect on the flow of intermediates if a pathway is blocked (for example, by a drug or an inherited deficiency of an enzyme). Throughout the next three units of this book, each pathway under discussion will be repeatedly featured as part of the major metabolic map shown in Figure 8.2.

B. Catabolic pathways

Catabolic reactions serve to capture chemical energy in the form of adenosine triphosphate (ATP) from the degradation of energy-rich fuel molecules. Catabolism also allows molecules in the diet (or nutrient molecules stored in cells) to be converted into building blocks needed for the synthesis of complex molecules. Energy generation by

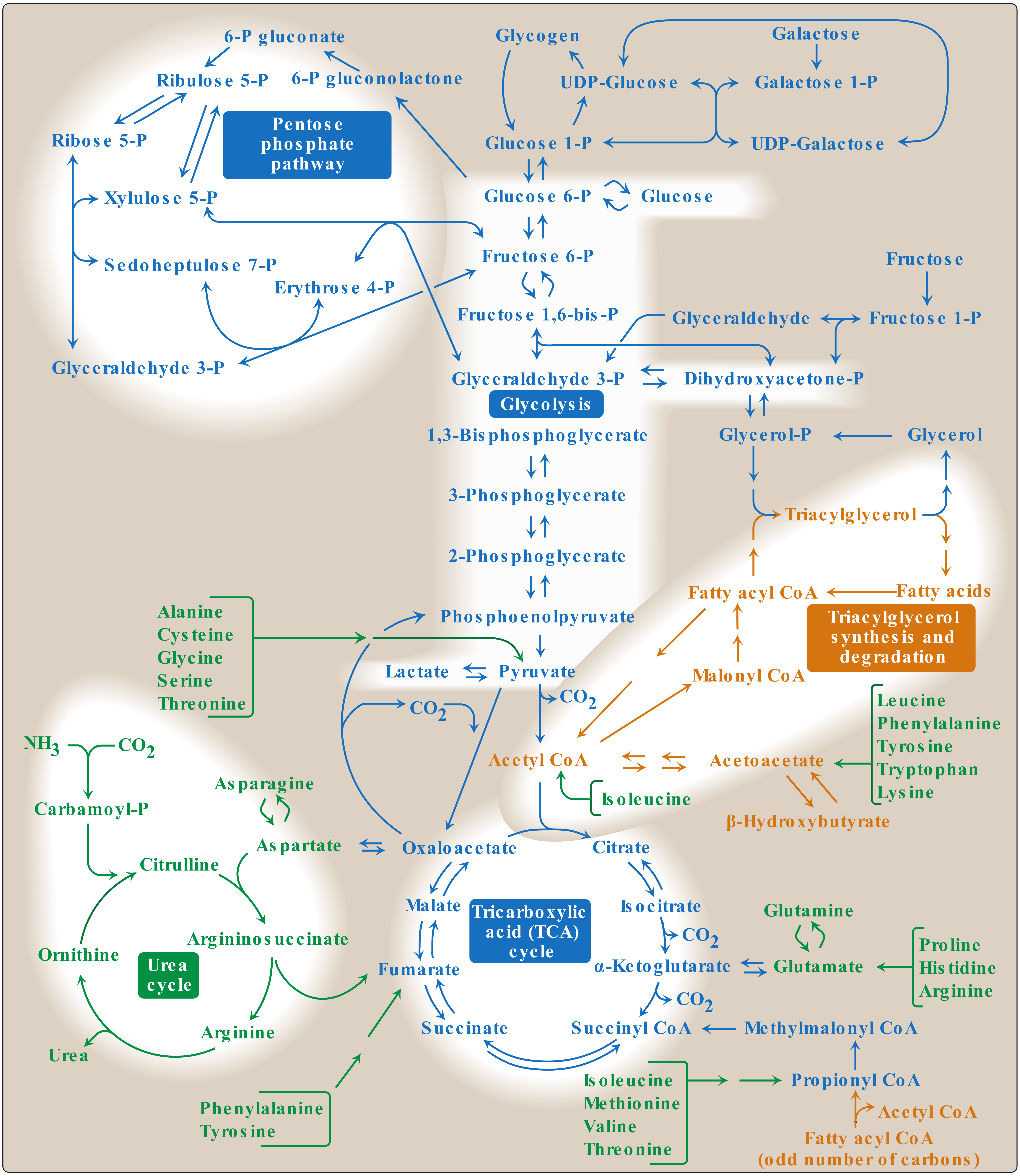

6-P gluconate
Ribulose 5-P
6-P gluconolactone
Ribose 5-P
Pentose phosphate pathway
Xylulose 5-P
Sedoheptulose 7-P
Erythrose 4-P
Glyceraldehyde 3-P
Glycogen
UDP-Glucose
Galactose
Galactose 1-P
Glucose 1-P
UDP-Galactose
Glucose 6-P
Glucose
Fructose 6-P
Fructose
Fructose 1,6-bis-P
Glyceraldehyde
Fructose 1-P
Glyceraldehyde 3-P
Dihydroxyacetone-P
Glycolysis
1,3-Bisphosphoglycerate
Glycerol-P
Glycerol
3-Phosphoglycerate
Triacylglycerol
2-Phosphoglycerate
Fatty acyl CoA
Fatty acids
Alanine
Cysteine
Glycine
Serine
Threonine
Phosphoenolpyruvate
Triacylglycerol synthesis and degradation
Lactate
Pyruvate
Malonyl CoA
CO_2
CO_2
Leucine
Phenylalanine
Tyrosine
Tryptophan
Lysine
NH_3
CO_2
Acetyl CoA
Acetoacetate
Carbamoyl-P
Asparagine
Isoleucine
β-Hydroxybutyrate
Aspartate
Oxaloacetate
Citrate
Citrulline
Malate
Tricarboxylic acid (TCA) cycle
Isocitrate
Glutamine
Argininosuccinate
CO_2
Ornithine
Urea cycle
Fumarate
α-Ketoglutarate
Glutamate
Proline
Histidine
Arginine
CO_2
Arginine
Succinate
Succinyl CoA
Methylmalonyl CoA
Urea
Isoleucine
Methionine
Valine
Threonine
Propionyl CoA
Phenylalanine
Tyrosine
Acetyl CoA
Fatty acyl CoA (odd number of carbons)

Figure 8.2

Important reactions of intermediary metabolism. Several important pathways to be discussed in later chapters are highlighted. Curved reaction arrows (⟲) indicate forward and reverse reactions that are catalyzed by different enzymes. The straight arrows (⇆) indicate forward and reverse reactions that are catalyzed by the same enzyme. **Blue text** = intermediates of carbohydrate metabolism; **brown text** = intermediates of lipid metabolism; **green text** = intermediates of protein metabolism. UDP = uridine diphosphate; P = phosphate; CoA = coenzyme A.

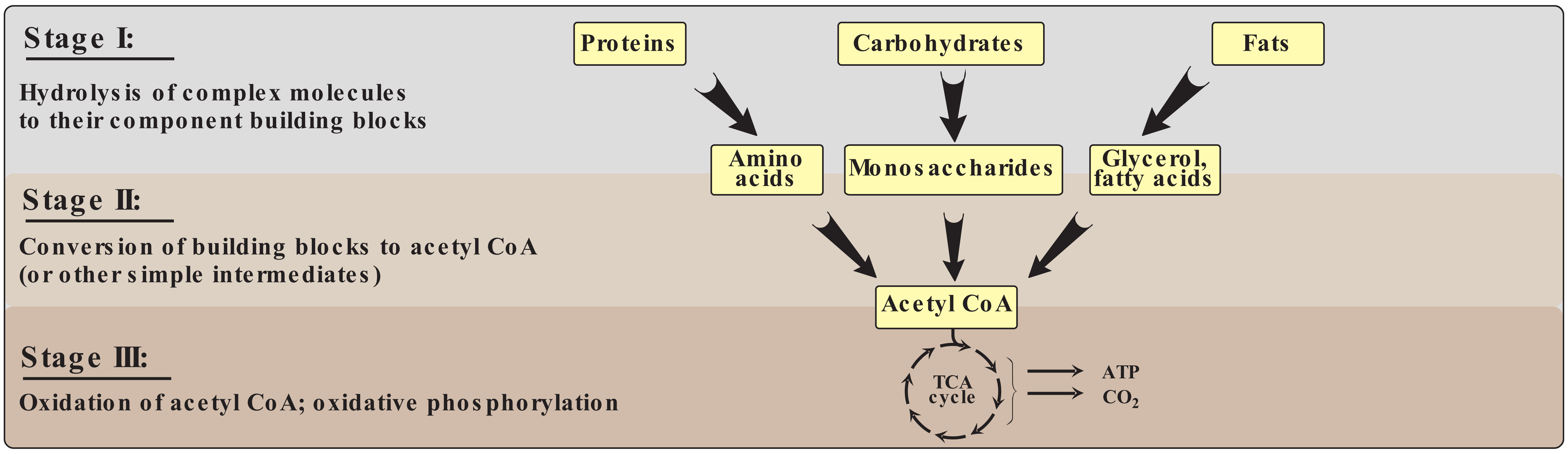

Stage I:
Hydrolysis of complex molecules to their component building blocks
Stage II:
Conversion of building blocks to acetyl CoA (or other simple intermediates)
Stage III:
Oxidation of acetyl CoA; oxidative phosphorylation
Proteins
Carbohydrates
Fats
Amino acids
Monosaccharides
Glycerol, fatty acids
Acetyl CoA
TCA cycle
ATP
CO₂

Figure 8.3
Three stages of catabolism. CoA = coenzyme A; TCA = tricarboxylic acid.

degradation of complex molecules occurs in three stages as shown in Figure 8.3. [Note: Catabolic pathways are typically oxidative, and require oxidized coenzymes such as nicotinamide adenine dinucleotide (NAD^+).]

1. **Hydrolysis of complex molecules:** In the first stage, complex molecules are broken down into their component building blocks. For example, proteins are degraded to amino acids, polysaccharides to monosaccharides, and fats (triacylglycerols) to free fatty acids and glycerol.

2. **Conversion of building blocks to simple intermediates:** In the second stage, these diverse building blocks are further degraded to acetyl coenzyme A (CoA) and a few other simple molecules. Some energy is captured as ATP, but the amount is small compared with the energy produced during the third stage of catabolism.

3. **Oxidation of acetyl coenzyme A:** The tricarboxylic acid (TCA) cycle (see p. 109) is the final common pathway in the oxidation of fuel molecules that produce acetyl CoA. Oxidation of acetyl CoA generates large amounts of ATP via oxidative phosphorylation as electrons flow from NADH and flavin adenine dinucleotide ($FADH_2$) to oxygen (see p. 73).

C. Anabolic pathways

Anabolic reactions combine small molecules, such as amino acids, to form complex molecules such as proteins (Figure 8.4). Anabolic reactions require energy (are endergonic), which is generally provided by the hydrolysis of ATP to adenosine diphosphate (ADP) and inorganic phosphate (P_i). Anabolic reactions often involve chemical reductions in which the reducing power is most frequently provided by the electron donor NADPH (see p. 147). Note that catabolism is a convergent process (that is, a wide variety of molecules are transformed into a few common end products). By contrast, anabolism is a divergent process in which a few biosynthetic precursors form a wide variety of polymeric, or complex, products.

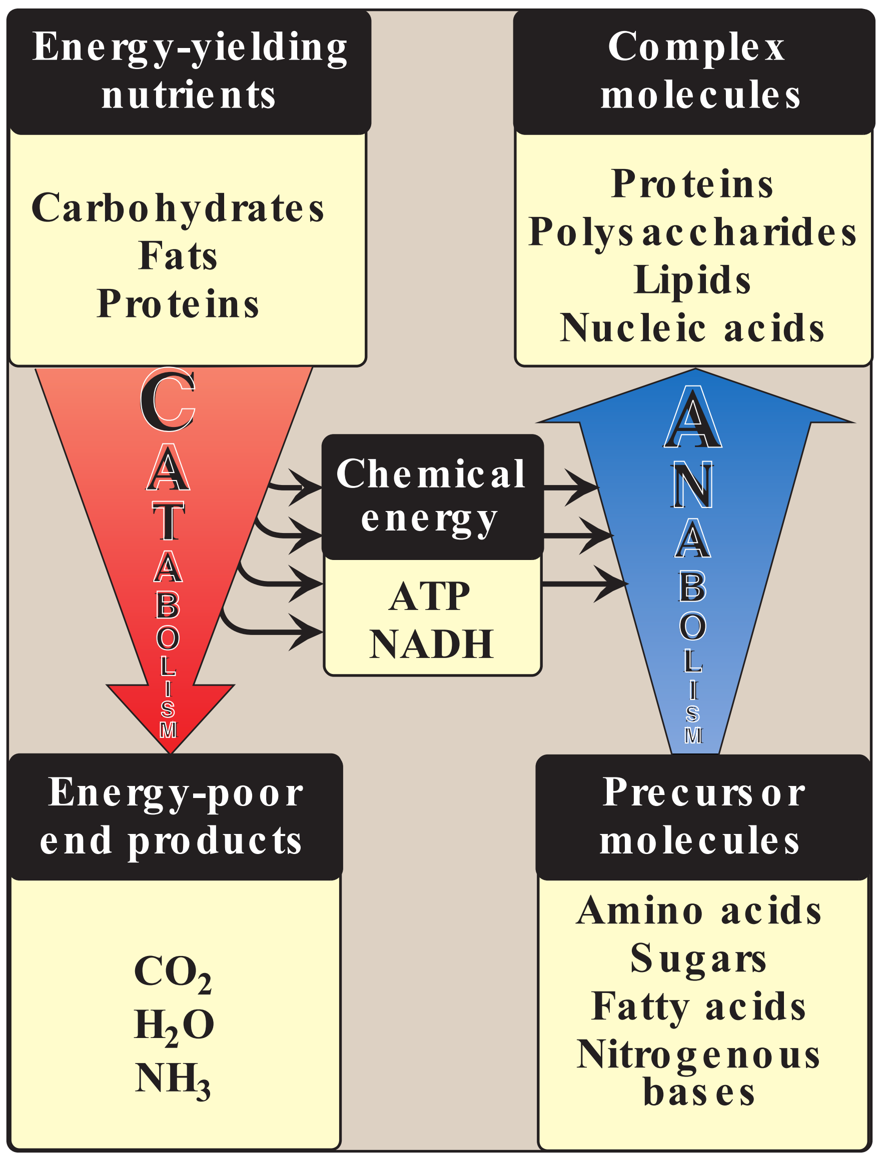
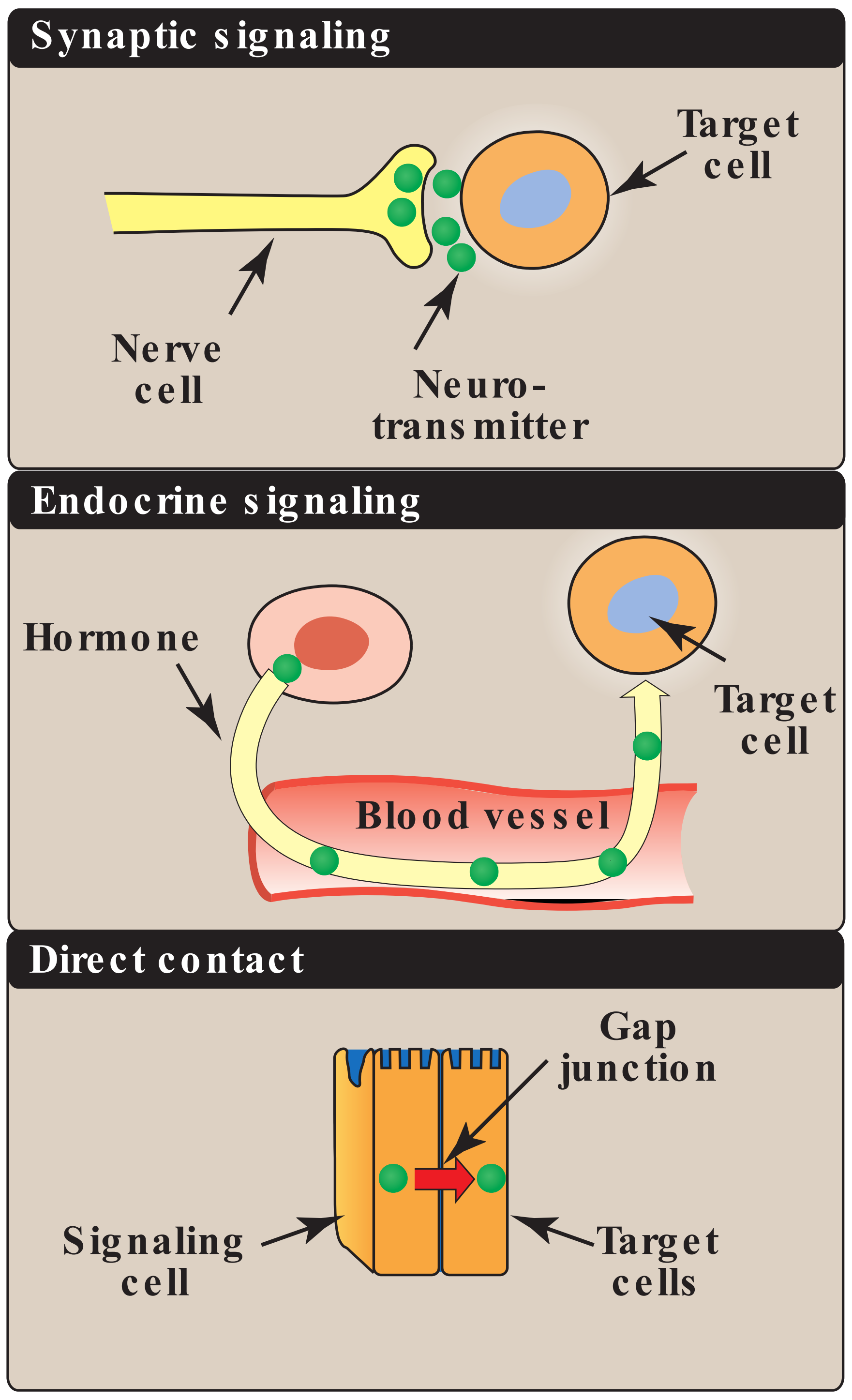

Energy-yielding nutrients
Carbohydrates
Fats
Proteins
Complex molecules
Proteins
Polysaccharides
Lipids
Nucleic acids
CATABOLISM
ANABOLISM
Chemical energy
ATP
NADH
Energy-poor end products
CO₂
H₂O
NH₃
Precursor molecules
Amino acids
Sugars
Fatty acids
Nitrogenous bases

Figure 8.4
Comparison of catabolic and anabolic pathways. ATP = adenosine triphosphate; NADH = nicotinamide adenine dinucleotide.

II. REGULATION OF METABOLISM

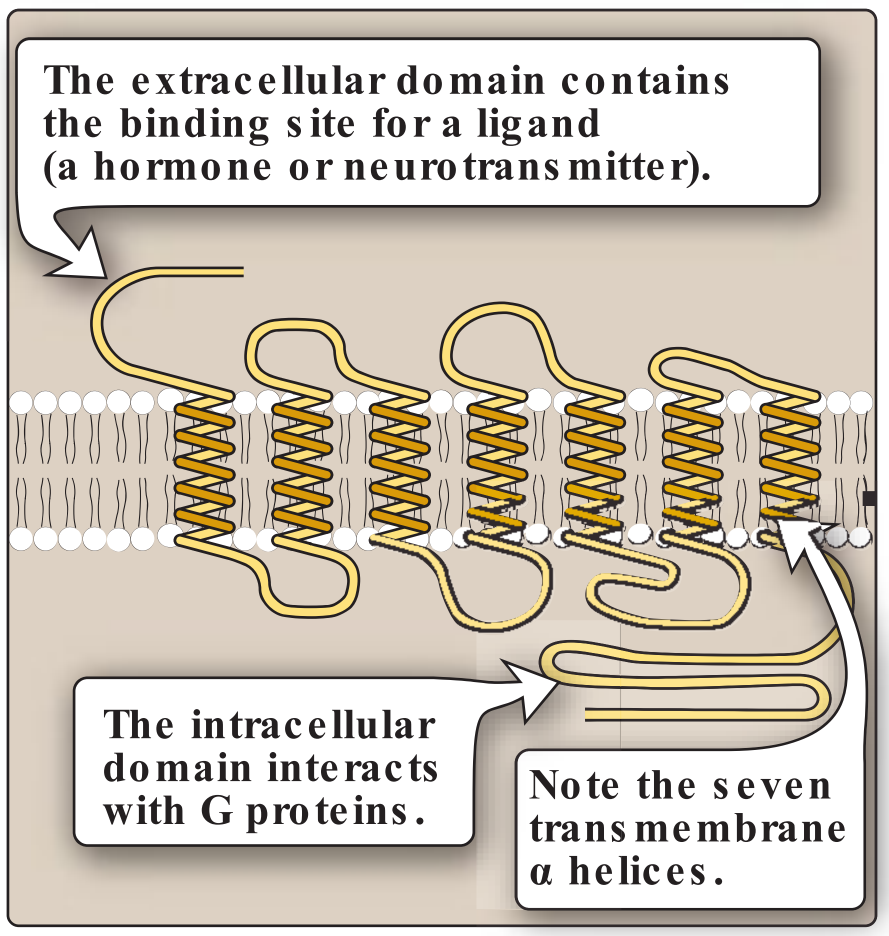
The pathways of metabolism must be coordinated so that the production of energy or the synthesis of end products meets the needs of the


Synaptic signaling

Endocrine signaling

Direct contact

Figure 8.5
Some commonly used mechanisms for transmission of regulatory signals between cells.


The extracellular domain contains the binding site for a ligand (a hormone or neurotransmitter).

The intracellular domain interacts with G proteins.

Note the seven transmembrane α helices.

Figure 8.6
Structure of a typical G protein–coupled receptor of the plasma membrane.

cell. Furthermore, individual cells do not function in isolation but, rather, are part of a community of interacting tissues. Thus, a sophisticated communication system has evolved to coordinate the functions of the body. Regulatory signals that inform an individual cell of the metabolic state of the body as a whole include hormones, neurotransmitters, and the availability of nutrients. These, in turn, influence signals generated within the cell (Figure 8.5).

A. Intracellular communication

The rate of a metabolic pathway can respond to regulatory signals that arise from within the cell. For example, the rate of a pathway may be influenced by the availability of substrates, product inhibition, or alterations in the levels of allosteric activators or inhibitors. These intracellular signals typically elicit rapid responses, and are important for the moment-to-moment regulation of metabolism.

B. Intercellular communication

The ability to respond to intercellular signals is essential for the development and survival of organisms. Signaling between cells provides for long-range integration of metabolism and usually results in a response, such as a change in gene expression, that is slower than is seen with intracellular signals. Communication between cells can be mediated, for example, by surface-to-surface contact and, in some tissues, by formation of gap junctions, allowing direct communication between the cytoplasms of adjacent cells. However, for energy metabolism, the most important route of communication is chemical signaling between cells by bloodborne hormones or by neurotransmitters.

C. Second messenger systems

Hormones or neurotransmitters can be thought of as signals and their receptors as signal detectors. Each component serves as a link in the communication between extracellular events and chemical changes within the cell. Many receptors signal their recognition of a bound ligand by initiating a series of reactions that ultimately result in a specific intracellular response. "Second messenger" molecules, so named because they intervene between the original messenger (the neurotransmitter or hormone) and the ultimate effect on the cell, are part of the cascade of events that translates (transduces) hormone or neurotransmitter binding into a cellular response. Two of the most widely recognized second messenger systems are the calcium/phosphatidylinositol system (see p. 205) and the adenylyl cyclase (adenylate cyclase) system, which is particularly important in regulating the pathways of intermediary metabolism.

D. Adenylyl cyclase

The recognition of a chemical signal by some plasma (cell) membrane receptors, such as the β- and α_2-adrenergic receptors, triggers either an increase or a decrease in the activity of adenylyl cyclase (AC). This is a membrane-bound enzyme that converts ATP to 3',5'-adenosine monophosphate (commonly called cyclic AMP, or cAMP). The chemical signals are most often hormones or neurotransmitters, each of which binds to a unique type of membrane receptor. Therefore, tissues that respond to more than one chemical signal must have several different receptors, each of which can be linked to AC. These receptors, known as G protein–coupled receptors (GPCRs), are characterized by an extracellular ligand-binding domain, seven transmembrane α helices, and an intracellular domain that interacts with G proteins (Figure 8.6).

1. **Guanosine triphosphate–dependent regulatory proteins:** The effect of the activated, occupied GPCR on second messenger formation is not direct but, rather, is mediated by specialized trimeric proteins (α, β, and γ subunits) of the cell membrane. These proteins, referred to as G proteins because the α subunit binds guanine nucleotides (GTP and GDP), form a link in the chain of communication between the receptor and AC. In the inactive form of a G protein, the α-subunit is bound to GDP (Figure 8.7). Binding of ligand causes a conformational change in the receptor, triggering replacement of this GDP with GTP. The GTP-bound form of the α subunit dissociates from the βγ subunits and moves to AC, which is thereby activated. Many molecules of active Gα protein are formed by one activated receptor. [Note: The ability of a hormone or neurotransmitter to stimulate or inhibit AC depends on the type of Gα protein that is linked to the receptor. One type, designated G_s, stimulates AC, whereas another type, designated G_i, inhibits the enzyme (not shown in Figure 8.7).] The actions of the Gα–GTP complex are short-lived because Gα has an inherent GTPase activity, resulting in the rapid hydrolysis of GTP to GDP. This causes inactivation of the Gα, its dissociation from AC, and reassociation with the βγ dimer.

Toxins from Vibrio cholerae (cholera) and Bordetella pertussis (whooping cough) cause inappropriate activation of adenylyl cyclase through covalent modification (ADP-ribosylation) of different G proteins. With cholera, the GTPase activity of $G\alpha_s$ is inhibited in intestinal cells. With whooping cough, $G\alpha_i$ is inactivated in respiratory-tract cells.

2. **Protein kinases:** The next key link in the cAMP second messenger system is the activation by cAMP of a family of enzymes called cAMP-dependent protein kinases such as protein kinase A (Figure 8.8). cAMP activates protein kinase A by binding to its two regulatory subunits, causing the release of two active, catalytic subunits. The active subunits catalyze the transfer of phosphate from ATP to specific serine or threonine residues of protein substrates. The phosphorylated proteins may act directly on the cell's ion channels or, if enzymes, may become activated or inhibited. Protein kinase A can also phosphorylate proteins that bind to DNA, causing changes in gene expression (see p. 456). [Note: Several types of protein kinases are not cAMP dependent, for example, protein kinase C described on p. 205.]

3. **Dephosphorylation of proteins:** The phosphate groups added to proteins by protein kinases are removed by protein phosphatases, enzymes that hydrolytically cleave phosphate esters (see Figure 8.8). This ensures that changes in protein activity induced by phosphorylation are not permanent.

4. **Hydrolysis of cyclic adenosine monophosphate:** cAMP is rapidly hydrolyzed to 5'-AMP by cAMP phosphodiesterase, one of a family of enzymes that cleave the cyclic 3',5'-phosphodiester bond.

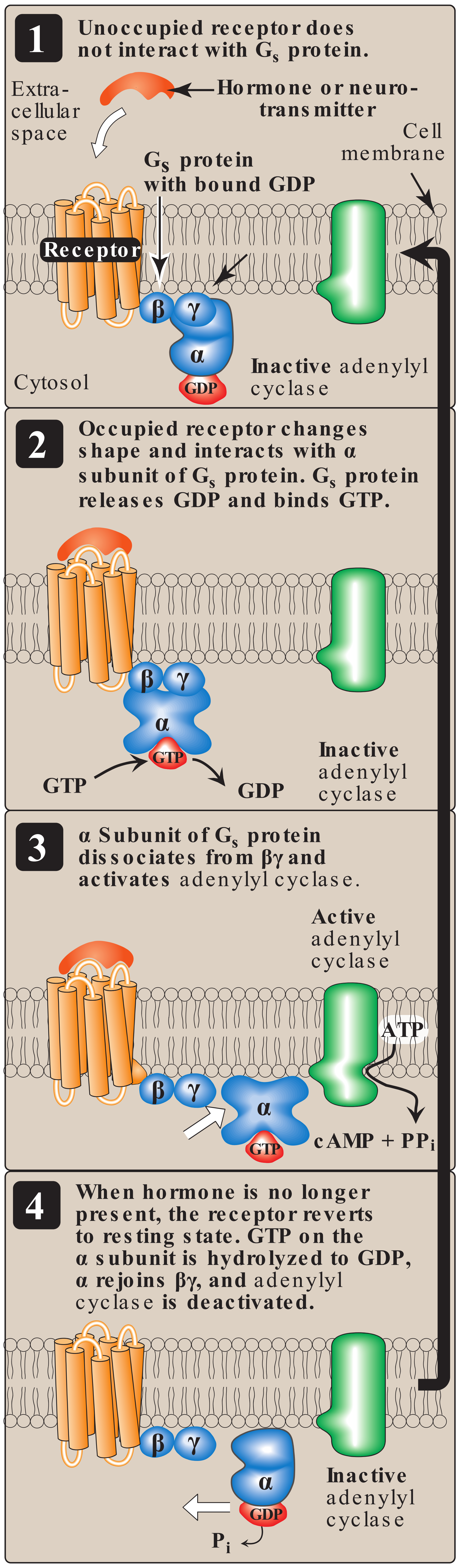

1
Unoccupied receptor does not interact with Gs protein.
Extra-cellular space
Hormone or neuro-transmitter
Cell membrane
Gs protein with bound GDP
Receptor
β γ α GDP
Cytosol
Inactive adenylyl cyclase
2
Occupied receptor changes shape and interacts with α subunit of Gs protein. Gs protein releases GDP and binds GTP.
β γ α GTP
GTP
GDP
Inactive adenylyl cyclase
3
α Subunit of Gs protein dissociates from βγ and activates adenylyl cyclase.
Active adenylyl cyclase
ATP
β γ α GTP
cAMP + PPi
4
When hormone is no longer present, the receptor reverts to resting state. GTP on the α subunit is hydrolyzed to GDP, α rejoins βγ, and adenylyl cyclase is deactivated.
β γ α GDP
Pi
Inactive adenylyl cyclase

Figure 8.7
The recognition of chemical signals by certain membrane receptors triggers an increase (or, less often, a decrease) in the activity of adenylyl cyclase. GDP = guanosine diphosphate; GTP = guanosine triphosphate; cAMP = cyclic AMP.

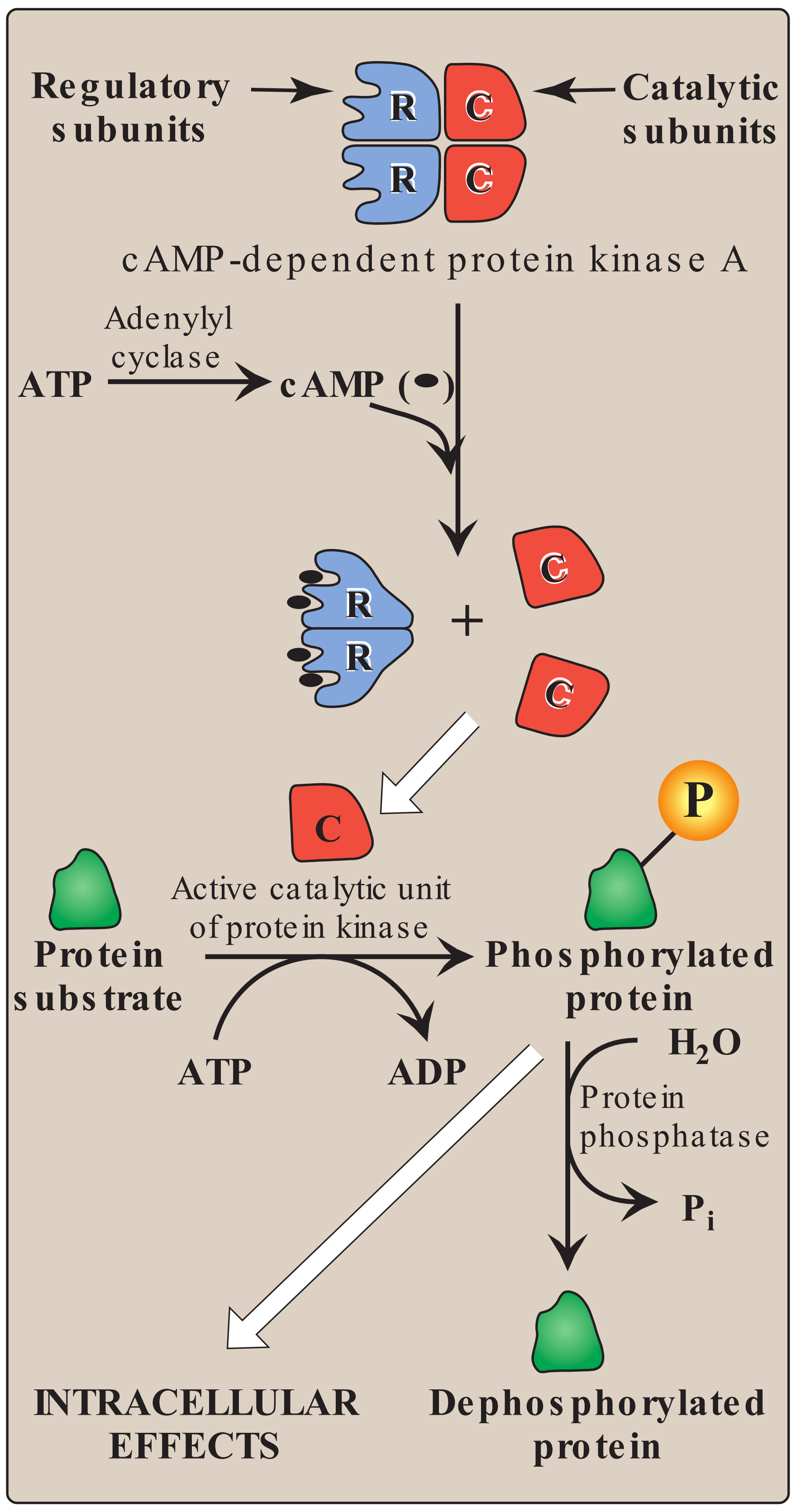

Regulatory subunits
Catalytic subunits
R
C
R
C
cAMP-dependent protein kinase A
Adenylyl cyclase
ATP
cAMP (●)
R
R
+
C
C
C
Active catalytic unit of protein kinase
P
Protein substrate
Phosphorylated protein
ATP
ADP
H_2O
Protein phosphatase
P_i
INTRACELLULAR EFFECTS
Dephosphorylated protein

Figure 8.8
Actions of cyclic AMP (cAMP). P_i = inorganic phosphate.

5'-AMP is not an intracellular signaling molecule. Therefore, the effects of neurotransmitter- or hormone-mediated increases of cAMP are rapidly terminated if the extracellular signal is removed. [Note: Phosphodiesterase is inhibited by the methylxanthine derivative, caffeine.]

III. OVERVIEW OF GLYCOLYSIS

The glycolytic pathway is employed by all tissues for the oxidation of glucose to provide energy (in the form of ATP) and intermediates for other metabolic pathways. Glycolysis is at the hub of carbohydrate metabolism because virtually all sugars, whether arising from the diet or from catabolic reactions in the body, can ultimately be converted to glucose (Figure 8.9A). Pyruvate is the end product of glycolysis in cells with mitochondria and an adequate supply of oxygen. This series of ten reactions is called aerobic glycolysis because oxygen is required to reoxidize the NADH formed during the oxidation of glyceraldehyde 3-phosphate (Figure 8.9B). Aerobic glycolysis sets the stage for the oxidative decarboxylation of pyruvate to acetyl CoA, a major fuel of the TCA cycle. Alternatively, pyruvate is reduced to lactate as NADH is oxidized to NAD^+ (Figure 8.9C). This conversion of glucose to lactate is called anaerobic glycolysis because it can occur without the participation of oxygen. Anaerobic glycolysis allows the production of ATP in tissues that lack mitochondria (for example, red blood cells and parts of the eye) or in cells deprived of sufficient oxygen.

IV. TRANSPORT OF GLUCOSE INTO CELLS

Glucose cannot diffuse directly into cells but enters by one of two transport mechanisms: a Na^+-independent, facilitated diffusion transport system or an ATP-dependent Na^+-monosaccharide cotransport system.

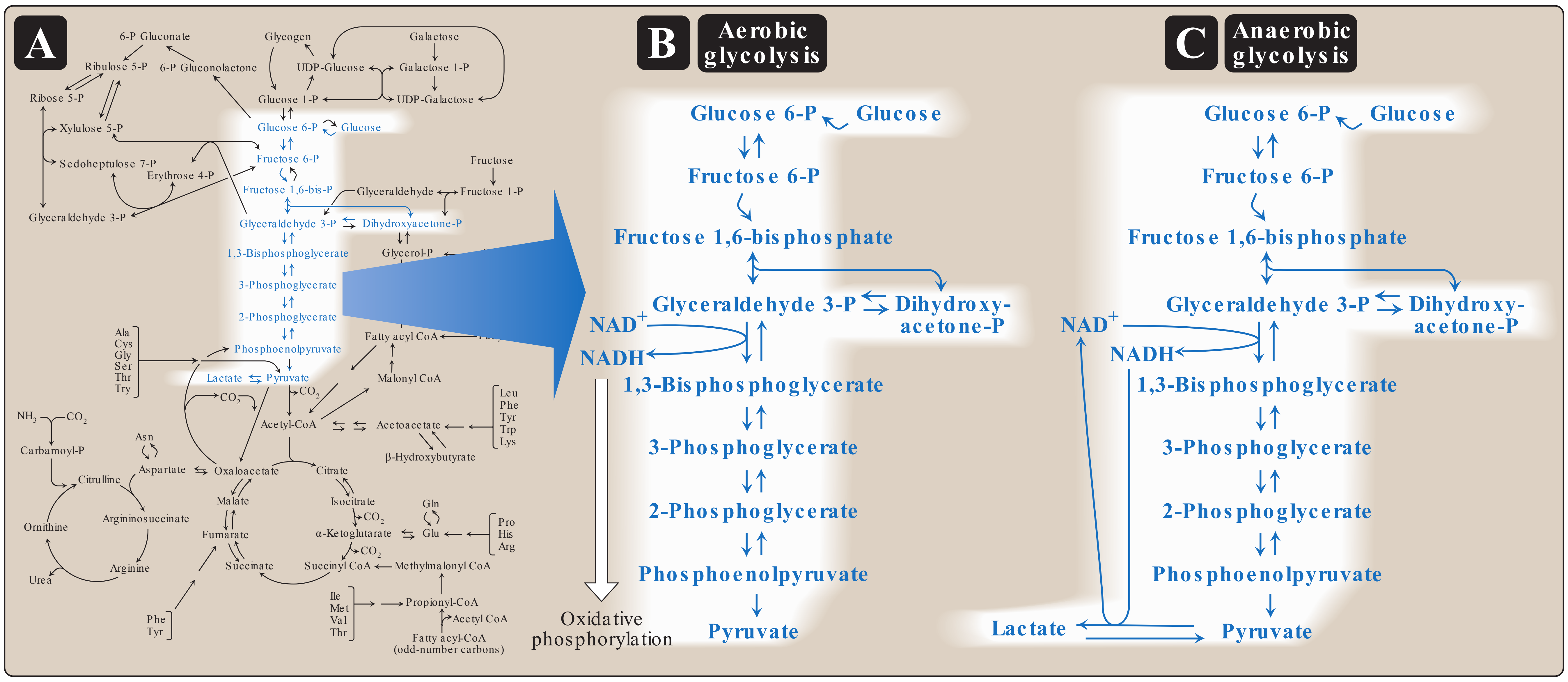

A
6-P Gluconate
Ribulose 5-P
6-P Gluconolactone
Glycogen
Galactose
UDP-Glucose
Galactose 1-P
Ribose 5-P
Glucose 1-P
UDP-Galactose
Xylulose 5-P
Glucose 6-P
Glucose
Sedoheptulose 7-P
Erythrose 4-P
Fructose 6-P
Fructose
Fructose 1,6-bis-P
Glyceraldehyde
Fructose 1-P
Glyceraldehyde 3-P
Dihydroxyacetone-P
1,3-Bisphosphoglycerate
Glycerol-P
3-Phosphoglycerate
2-Phosphoglycerate
Ala
Cys
Gly
Ser
Thr
Try
Phosphoenolpyruvate
Fatty acyl CoA
Lactate
Pyruvate
Malonyl CoA
CO_2
NH_3
Carbamoyl-P
Acetyl-CoA
Acetoacetate
Leu
Phe
Tyr
Trp
Lys
β-Hydroxybutyrate
Asn
Aspartate
Oxaloacetate
Citrate
Citrulline
Malate
Isocitrate
Gln
Argininosuccinate
Ornithine
Fumarate
α-Ketoglutarate
Glu
Pro
His
Arg
Arginine
Succinate
Succinyl CoA
Methylmalonyl CoA
Urea
Ile
Met
Val
Thr
Propionyl-CoA
Acetyl CoA
Fatty acyl-CoA (odd-number carbons)
B
Aerobic glycolysis
Glucose 6-P
Glucose
Fructose 6-P
Fructose 1,6-bisphosphate
Glyceraldehyde 3-P
Dihydroxy-acetone-P
NAD^+
NADH
1,3-Bisphosphoglycerate
3-Phosphoglycerate
2-Phosphoglycerate
Phosphoenolpyruvate
Pyruvate
Oxidative phosphorylation
C
Anaerobic glycolysis
Glucose 6-P
Glucose
Fructose 6-P
Fructose 1,6-bisphosphate
Glyceraldehyde 3-P
Dihydroxy-acetone-P
NAD^+
NADH
1,3-Bisphosphoglycerate
3-Phosphoglycerate
2-Phosphoglycerate
Phosphoenolpyruvate
Lactate
Pyruvate

Figure 8.9
A. Glycolysis shown as one of the essential pathways of energy metabolism. B. Reactions of aerobic glycolysis. C. Reactions of anaerobic glycolysis. NAD(H) = nicotinamide adenine dinucleotide; P = phosphate.

A. Sodium-independent facilitated diffusion transport system

This system is mediated by a family of 14 glucose transporters found in cell membranes. They are designated GLUT-1 to GLUT-14 (glucose transporter isoforms 1–14). These monomeric protein transporters exist in the membrane in two conformational states (Figure 8.10). Extracellular glucose binds to the transporter, which then alters its conformation, transporting glucose across the cell membrane.

1. **Tissue specificity of glucose transporter gene expression:** The GLUTs display a tissue-specific pattern of expression. For example, GLUT-3 is the primary glucose transporter in neurons. GLUT-1 is abundant in erythrocytes and the blood–brain barrier but is low in adult muscle, whereas GLUT-4 is abundant in muscle and adipose tissue. [Note: The number of GLUT-4 transporters active in these tissues is increased by insulin. (See p. 311 for a discussion of insulin and glucose transport.)] GLUT-2 is abundant in liver, kidney, and β cells of the pancreas. The other GLUT isoforms also have tissue-specific distributions.

2. **Specialized functions of glucose transporter isoforms:** In facilitated diffusion, transporter-mediated glucose movement is down a concentration gradient (that is, from a high glucose concentration to a lower one and, therefore, does not require energy). For example, GLUT-1, GLUT-3, and GLUT-4 are primarily involved in glucose uptake from the blood. In contrast, GLUT-2, in the liver and kidney, can either transport glucose into these cells when blood glucose levels are high or transport glucose from these cells when blood glucose levels are low (for example, during fasting). GLUT-5 is unusual in that it is the primary transporter for fructose (not glucose) in the small intestine and the testes.

B. Sodium–monosaccharide cotransport system

This is an energy-requiring process that transports glucose “against” a concentration gradient (that is, from low glucose concentrations outside the cell to higher concentrations within the cell). This system is a transporter-mediated process in which the movement of glucose is coupled to the concentration gradient of Na^+, which is transported into the cell at the same time. The transporter is a sodium-dependent glucose transporter (SGLT). This type of transport occurs in the epithelial cells of the intestine (see p. 87), renal tubules, and choroid plexus. [Note: The choroid plexus, part of the blood–brain barrier, also contains GLUT-1.]

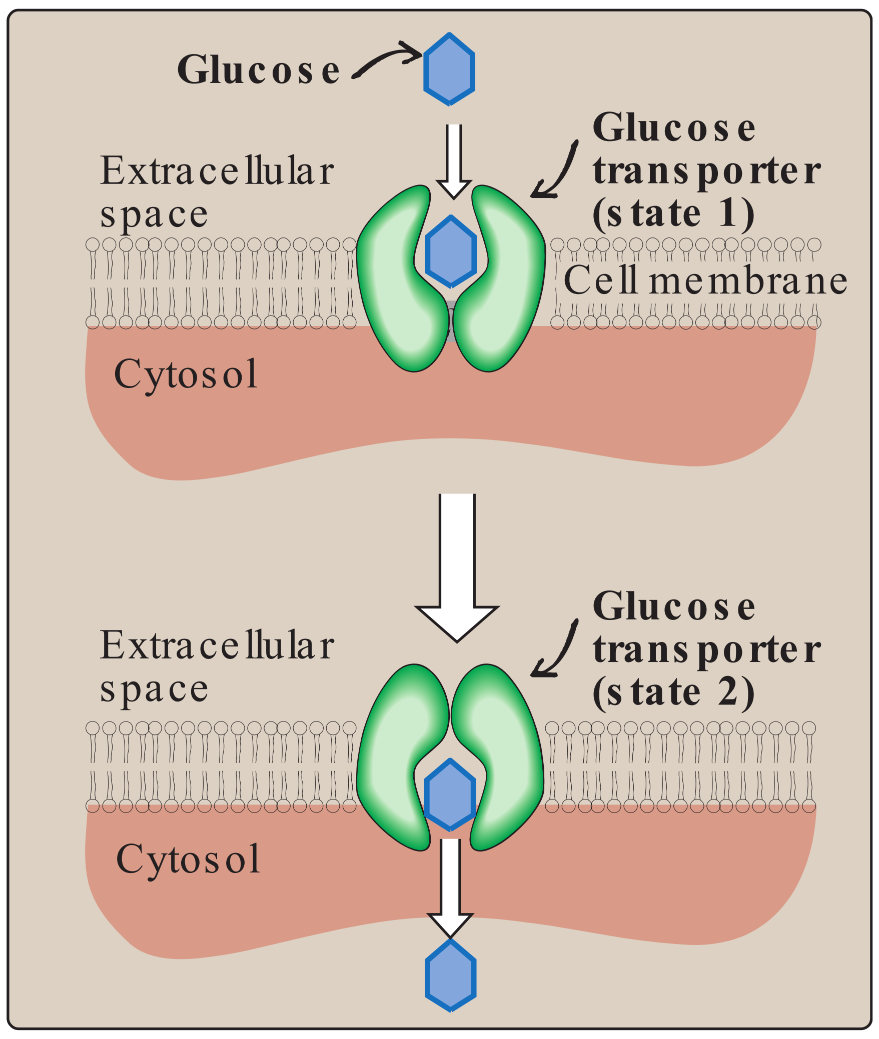


Figure 8.10
Schematic representation of the facilitated transport of glucose through a cell membrane. [Note: Glucose transporter proteins are monomeric and contain 12 transmembrane α helices.]

V. REACTIONS OF GLYCOLYSIS

The conversion of glucose to pyruvate occurs in two stages (Figure 8.11). The first five reactions of glycolysis correspond to an energy–investment phase in which the phosphorylated forms of intermediates are synthesized at the expense of ATP. The subsequent reactions of glycolysis constitute an energy–generation phase in which a net of two molecules of ATP are formed by substrate-level phosphorylation (see p. 102) per glucose molecule metabolized.

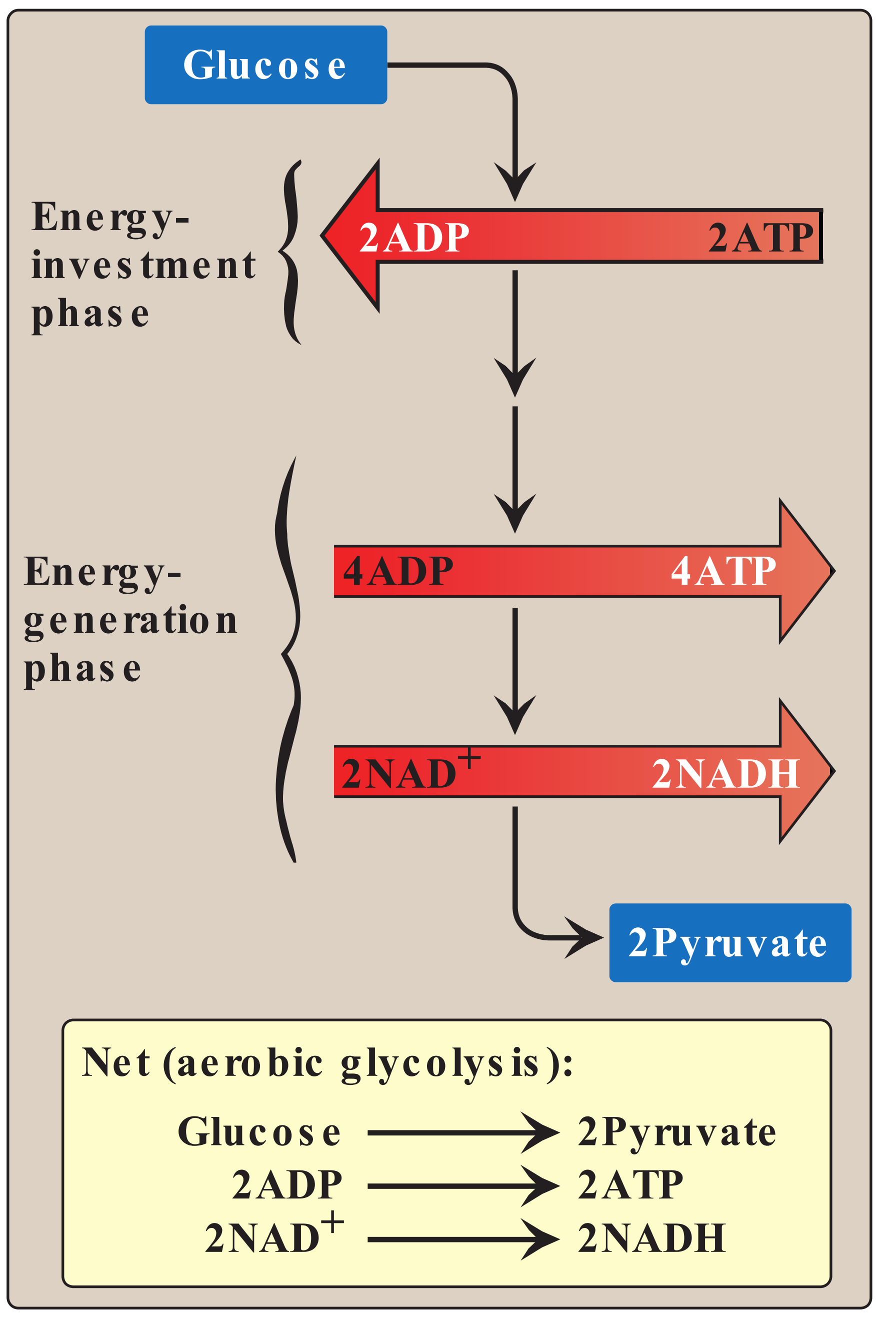


Figure 8.11
Two phases of aerobic glycolysis. NAD(H) = nicotinamide adenine dinucleotide.

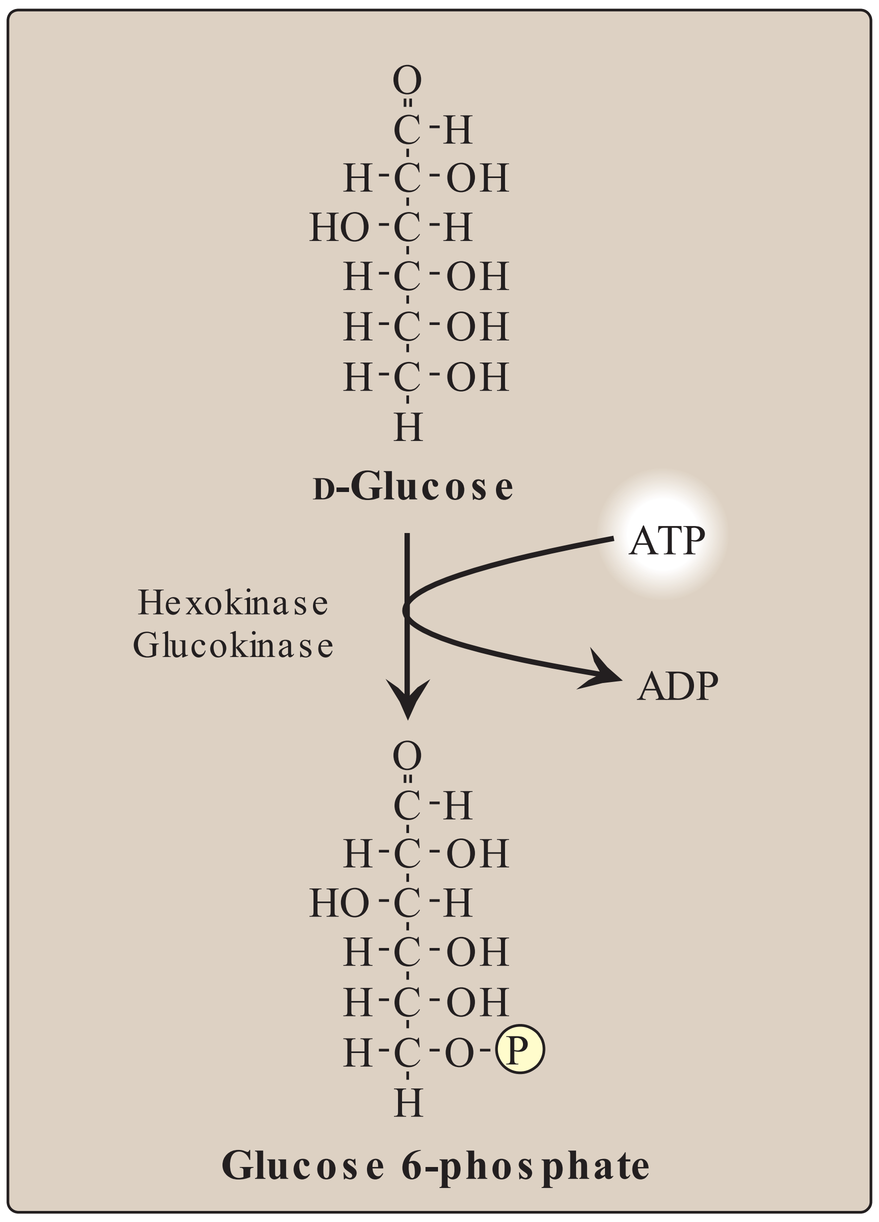


Figure 8.12
Energy-investment phase: phosphorylation of glucose. [Note: Kinases utilize ATP complexed with a divalent metal ion, most typically Mg^{2+}.]

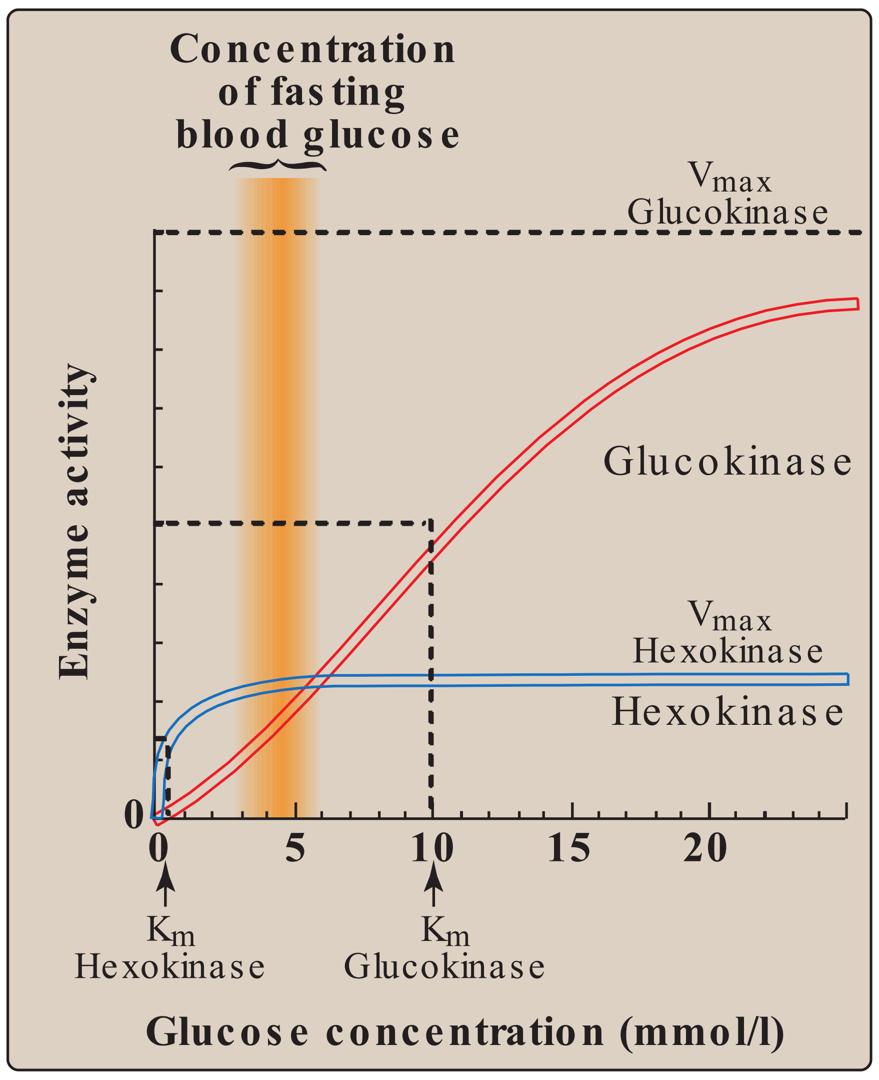


Figure 8.13
Effect of glucose concentration on the rate of phosphorylation catalyzed by hexokinase and glucokinase. K_m = Michaelis constant; V_{max} = maximal velocity.

A. Phosphorylation of glucose

Phosphorylated sugar molecules do not readily penetrate cell membranes because there are no specific transmembrane carriers for these compounds and because they are too polar to diffuse through the lipid core of membranes. The irreversible phosphorylation of glucose (Figure 8.12), therefore, effectively traps the sugar as cytosolic glucose 6-phosphate, thereby committing it to further metabolism in the cell. Mammals have four (I–IV) isozymes of the enzyme hexokinase that catalyze the phosphorylation of glucose to glucose 6-phosphate.

1. **Hexokinases I–III:** In most tissues, phosphorylation of glucose is catalyzed by one of these isozymes of hexokinase, which is one of three regulatory enzymes of glycolysis (see also phosphofructokinase and pyruvate kinase). These isozymes have broad substrate specificity and are able to phosphorylate several hexoses in addition to glucose. They are inhibited by the reaction product, glucose 6-phosphate, which accumulates when further metabolism of this hexose phosphate is reduced. Hexokinases I-III have a low Michaelis constant (K_m) (and, therefore, a high affinity; see p. 59) for glucose. This permits the efficient phosphorylation and subsequent metabolism of glucose even when tissue concentrations of glucose are low (Figure 8.13). These isozymes, however, have a low maximal velocity ([V_{max}] see p. 59) for glucose and, therefore, do not sequester (trap) cellular phosphate in the form of phosphorylated hexoses, or phosphorylate more sugars than the cell can use.

2. **Hexokinase IV (or, glucokinase):** In liver parenchymal cells and β cells of the pancreas, glucokinase (the hexokinase IV isozyme) is the predominant enzyme responsible for the phosphorylation of glucose. In β cells, glucokinase functions as a glucose sensor, determining the threshold for insulin secretion (see p. 309). [Note: Hexokinase IV also serves as a glucose sensor in neurons of the hypothalamus, playing a key role in the adrenergic response to hypoglycemia (see p. 315.] In the liver, the enzyme facilitates glucose phosphorylation during hyperglycemia. Despite the popular but misleading name glucokinase, the sugar specificity of the enzyme is similar to that of other hexokinase isozymes.

   a. **Kinetics:** Glucokinase differs from hexokinases I–III in several important properties. For example, it has a much higher K_m, requiring a higher glucose concentration for half-saturation (see Figure 8.13). Thus, glucokinase functions only when the intracellular concentration of glucose in the hepatocyte is elevated such as during the brief period following consumption of a carbohydrate-rich meal, when high levels of glucose are delivered to the liver via the portal vein. Glucokinase has a high V_{max}, allowing the liver to effectively remove the flood of glucose delivered by the portal blood. This prevents large amounts of glucose from entering the systemic circulation following such a meal thereby minimizing hyperglycemia during the absorptive period. [Note: GLUT-2 insures that blood glucose equilibrates rapidly across the membrane of the hepatocyte.]

   b. **Regulation by fructose 6-phosphate and glucose:** Glucokinase activity is not directly inhibited by glucose 6-phosphate as are the other hexokinases but, rather, is indirectly inhibited by fructose 6-phosphate (which is in equilibrium with glucose

6-phosphate, a product of glucokinase) and is indirectly stimulated by glucose (a substrate of glucokinase) via the following mechanism. Glucokinase regulatory protein (GKRP) in the liver regulates the activity of glucokinase through reversible binding. In the presence of fructose 6-phosphate, glucokinase is translocated into the nucleus and binds tightly to the regulatory protein, thereby rendering the enzyme inactive (Figure 8.14). When glucose levels in the blood (and also in the hepatocyte, as a result of GLUT-2) increase, glucokinase is released from the regulatory protein, and the enzyme reenters the cytosol where it phosphorylates glucose to glucose 6-phosphate. [Note: Fructose 1-phosphate inhibits formation of the glucokinase–GKRP complex.]

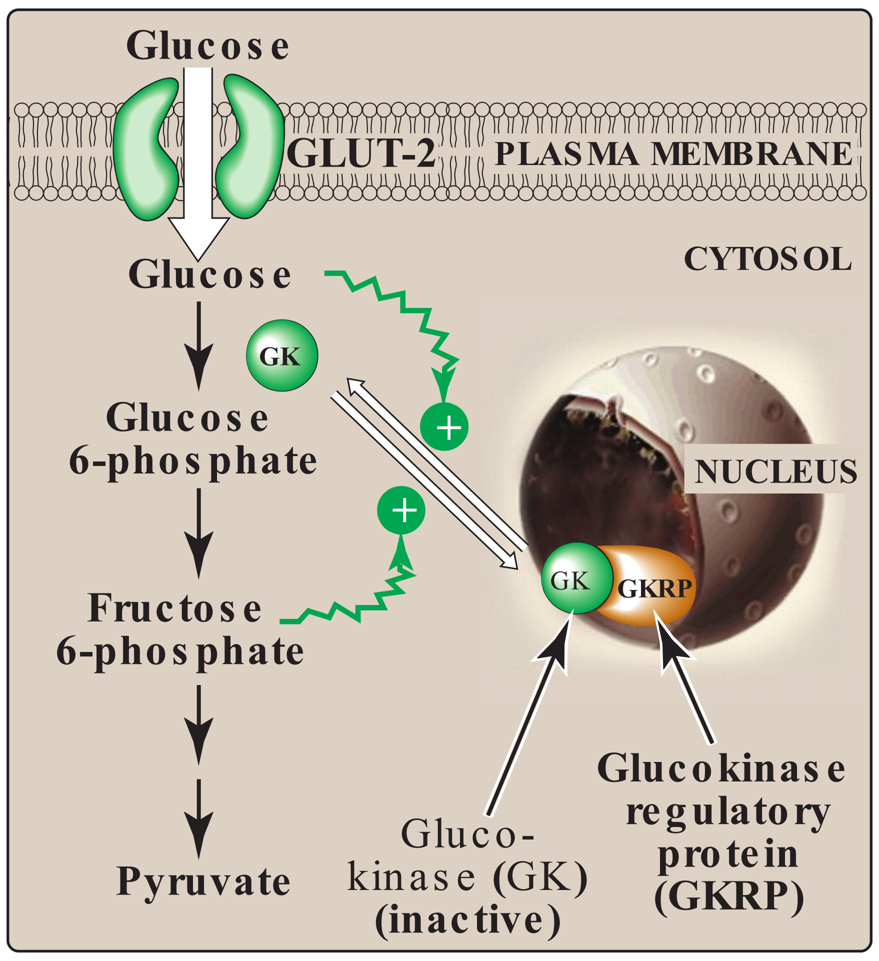


Figure 8.14
Regulation of glucokinase activity by glucokinase regulatory protein. GLUT = glucose transporter.

> Glucokinase functions as a glucose sensor in the maintenance of blood glucose homeostasis. Inactivating mutations of glucokinase are the cause of a rare form of diabetes, maturity onset diabetes of the young type 2 (MODY 2) that is characterized by impaired insulin secretion.

B. Isomerization of glucose 6-phosphate

The isomerization of glucose 6-phosphate to fructose 6-phosphate is catalyzed by phosphoglucose isomerase (Figure 8.15). The reaction is readily reversible and is not a rate-limiting or regulated step.

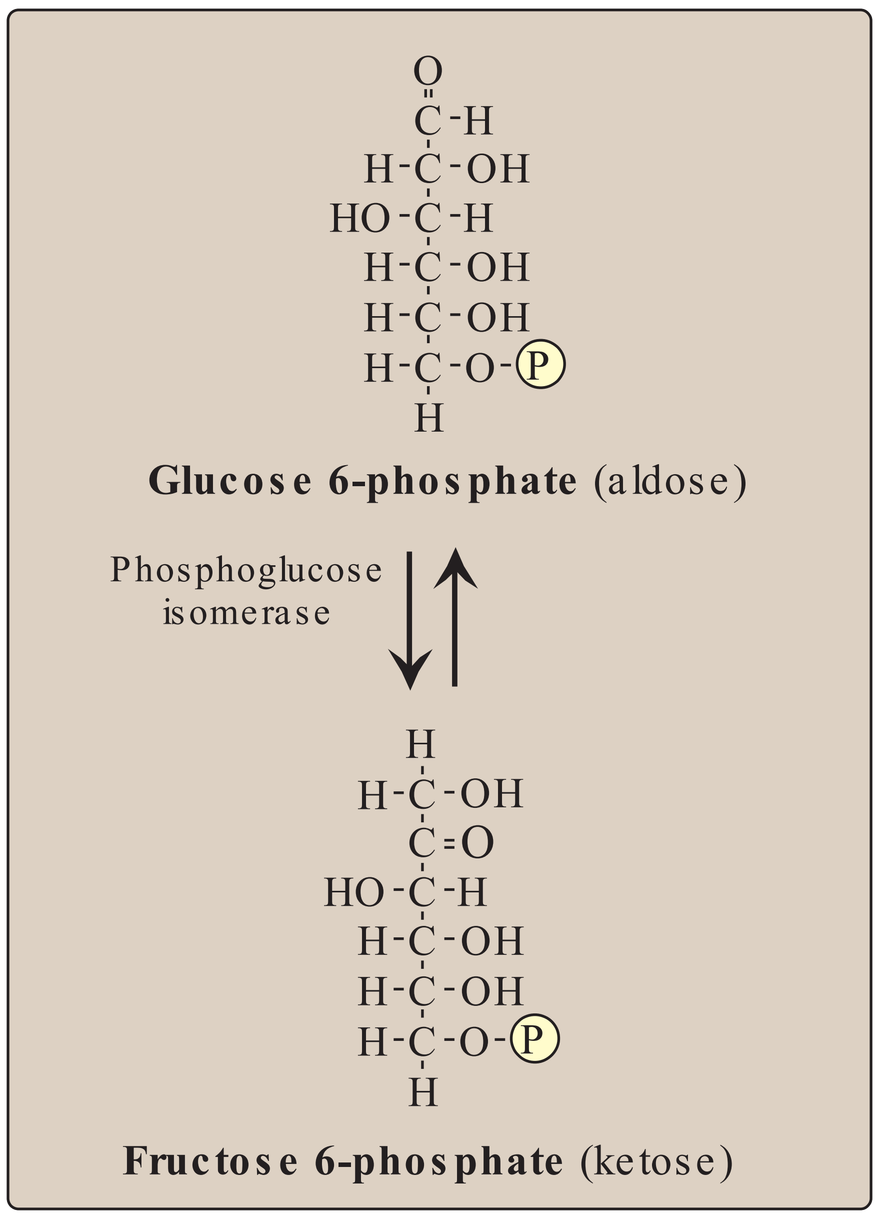


Figure 8.15
Aldose-ketose isomerization of glucose 6-phosphate to fructose 6-phosphate. P = phosphate.

C. Phosphorylation of fructose 6-phosphate

The irreversible phosphorylation reaction catalyzed by phosphofructokinase-1 (PFK-1) is the most important control point and the rate-limiting and committed step of glycolysis (Figure 8.16). PFK-1 is controlled by the available concentrations of the substrates ATP and fructose 6-phosphate as well as by regulatory substances described below.

1. **Regulation by energy levels within the cell:** PFK-1 is inhibited allosterically by elevated levels of ATP, which act as an "energy-rich" signal indicating an abundance of high-energy compounds. Elevated levels of citrate, an intermediate in the TCA cycle (see p. 109), also inhibit PFK-1. [Note: Inhibition by citrate favors the use of glucose for glycogen synthesis (see p. 125).] Conversely, PFK-1 is activated allosterically by high concentrations of AMP, which signal that the cell's energy stores are depleted.

2. **Regulation by fructose 2,6-bisphosphate:** Fructose 2,6-bisphosphate is the most potent activator of PFK-1 (see Figure 8.16) and is able to activate the enzyme even when ATP levels are high. Fructose 2,6-bisphosphate is formed from fructose 6-phosphate by phosphofructokinase-2 (PFK-2), an enzyme different than PFK-1. PFK-2 is a bifunctional protein that has both the kinase activity that produces fructose 2,6-bisphosphate and the phosphatase activity that dephosphorylates fructose 2,6-bisphosphate back to fructose 6-phosphate. In the liver, the kinase domain is active if dephosphorylated and is inactive if phosphorylated (Figure 8.17). [Note: Fructose 2,6-bisphosphate is an inhibitor

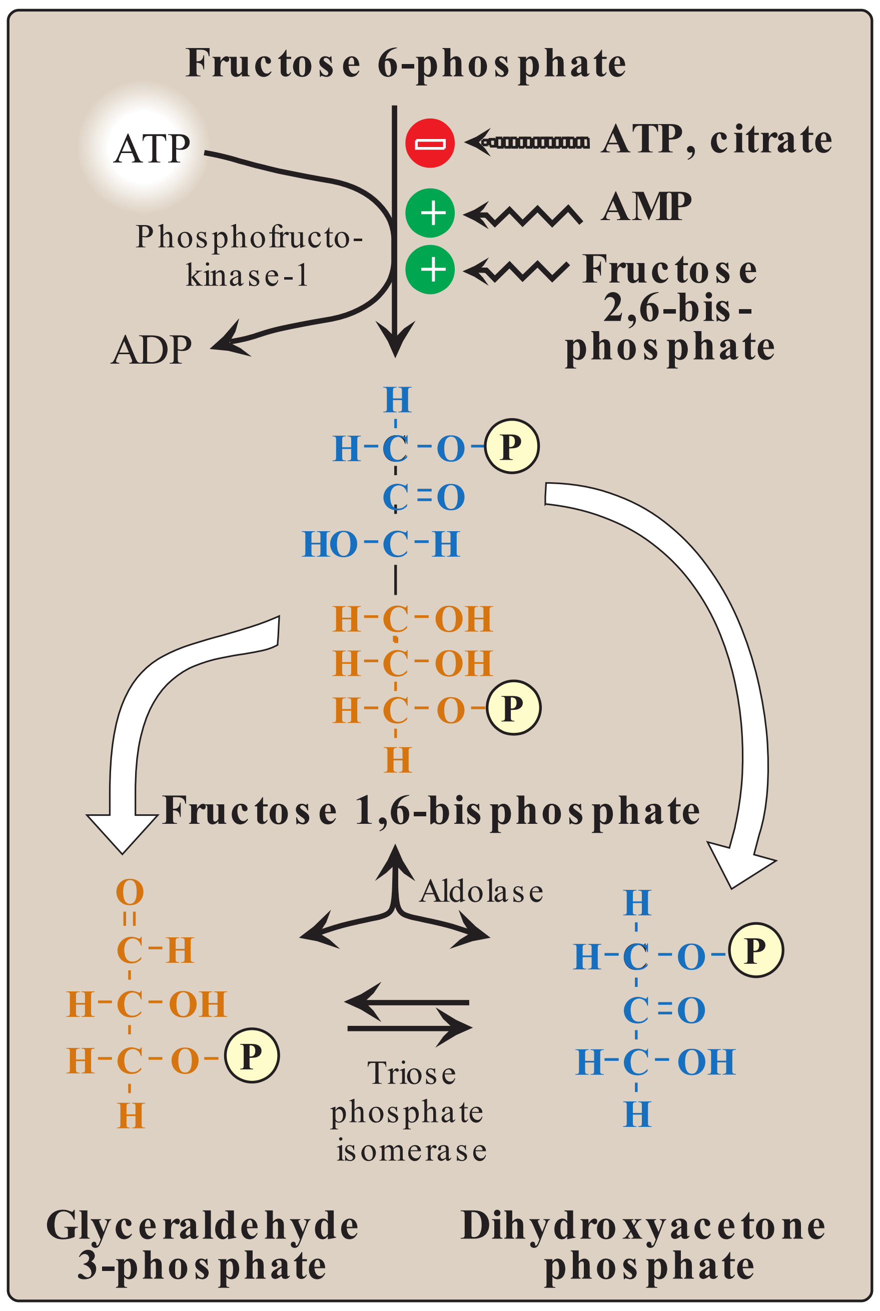

Fructose 6-phosphate
ATP
ATP, citrate
AMP
Fructose 2,6-bis-phosphate
Phosphofructo-kinase-1
ADP
H
H-C-O-P
C=O
HO-C-H
H-C-OH
H-C-OH
H-C-O-P
H
Fructose 1,6-bisphosphate
Aldolase
O
C-H
H-C-OH
H-C-O-P
H
Triose phosphate isomerase
H
H-C-O-P
C=O
H-C-OH
H
Glyceraldehyde 3-phosphate
Dihydroxyacetone phosphate

Figure 8.16
Energy–investment phase (continued): Conversion of fructose 6-phosphate to triose phosphates. P = phosphate; AMP = adenosine monophosphate.

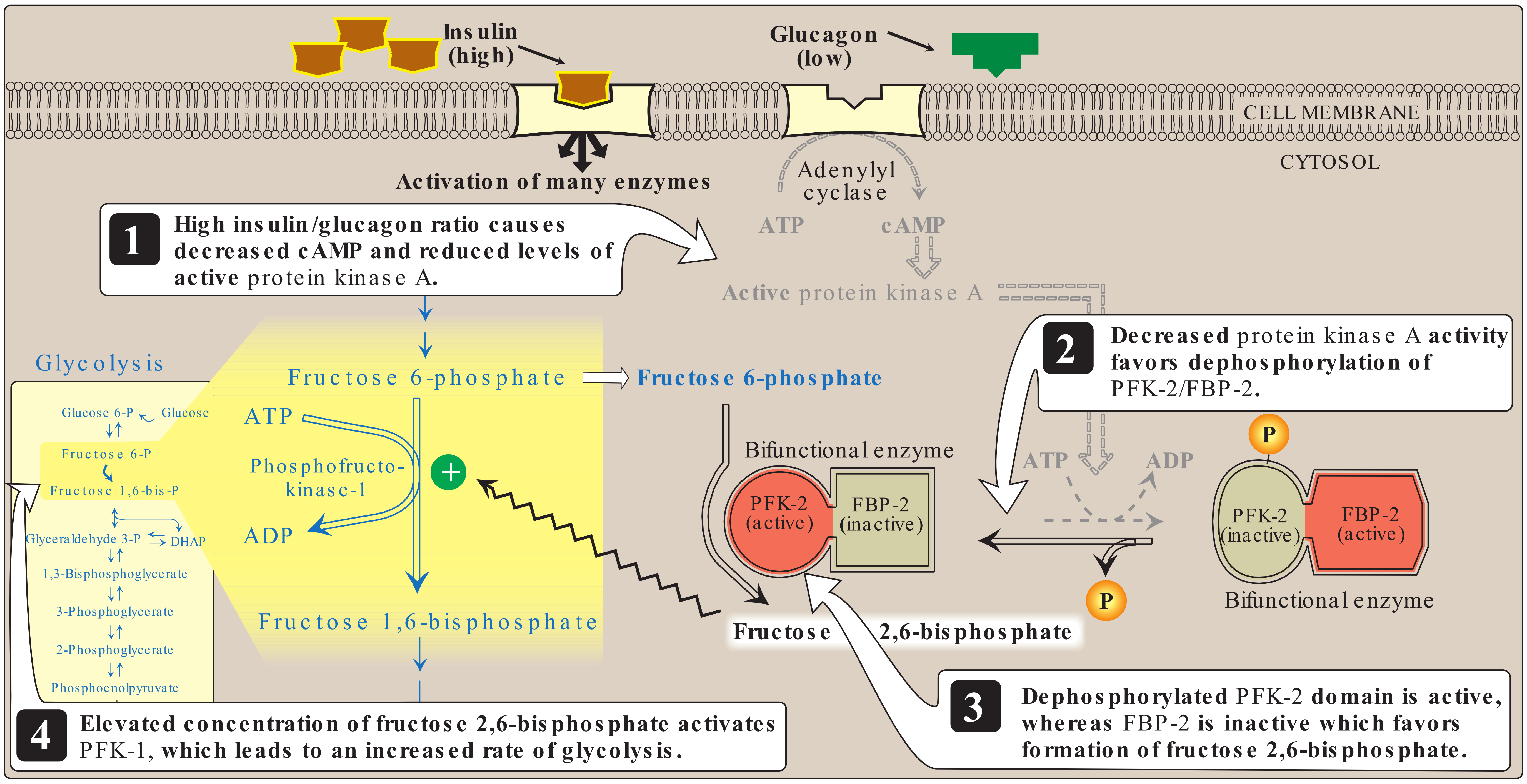
of fructose 1,6-bisphosphatase, an enzyme of gluconeogenesis (see p. 120). The reciprocal actions of fructose 2,6-bisphosphate on glycolysis (activation) and gluconeogenesis (inhibition) ensure that both pathways are not fully active at the same time, preventing a futile cycle in which glucose would be converted to pyruvate followed by resynthesis of glucose from pyruvate.]

a. **During the well-fed state:** Decreased levels of glucagon and elevated levels of insulin, such as occur following a carbohydrate-rich meal, cause an increase in fructose 2,6-bisphosphate and, thus, in the rate of glycolysis in the liver (see Figure 8.17). Fructose 2,6-bisphosphate, therefore, acts as an intracellular signal, indicating that glucose is abundant.

b. **During fasting:** Elevated levels of glucagon and low levels of insulin, such as occur during fasting (see p. 327), decrease the intracellular concentration of hepatic fructose 2,6-bisphosphate. This results in inhibition of glycolysis and activation of gluconeogenesis.

D. Cleavage of fructose 1,6-bisphosphate

Aldolase cleaves fructose 1,6-bisphosphate to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate (see Figure 8.16). The reaction is reversible and not regulated. [Note: Aldolase B, the isoform found primarily in the liver, also cleaves fructose 1-phosphate and functions in the metabolism of dietary fructose (see p. 138).]


Insulin (high)
Glucagon (low)
CELL MEMBRANE
CYTOSOL
Activation of many enzymes
Adenylyl cyclase
ATP
cAMP
Active protein kinase A
Glycolysis
Glucose 6-P
Glucose
Fructose 6-P
Fructose 1,6-bis-P
Glyceraldehyde 3-P
DHAP
1,3-Bisphosphoglycerate
3-Phosphoglycerate
2-Phosphoglycerate
Phosphoenolpyruvate
Fructose 6-phosphate
ATP
Phosphofructo-kinase-1
ADP
Fructose 1,6-bisphosphate
Fructose 6-phosphate
Bifunctional enzyme
PFK-2 (active)
FBP-2 (inactive)
ATP
ADP
P
P
PFK-2 (inactive)
FBP-2 (active)
Bifunctional enzyme
Fructose 2,6-bisphosphate

1 **High insulin/glucagon ratio causes decreased cAMP and reduced levels of active** protein kinase A.

2 **Decreased** protein kinase A **activity favors dephosphorylation of** PFK-2/FBP-2.

3 **Dephosphorylated** PFK-2 **domain is active, whereas** FBP-2 **is inactive which favors formation of fructose 2,6-bisphosphate.**

4 **Elevated concentration of fructose 2,6-bisphosphate activates** PFK-1, **which leads to an increased rate of glycolysis.**

Figure 8.17
Effect of elevated insulin concentration on the intracellular concentration of fructose 2,6-bisphosphate in liver. PFK-2 = phosphofructokinase-2; FBP-2 = fructose 2,6-bisphosphatase; cAMP = cyclic AMP; P = phosphate.

E. Isomerization of dihydroxyacetone phosphate

Triose phosphate isomerase interconverts dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (see Figure 8.16). DHAP must be isomerized to glyceraldehyde 3-phosphate for further metabolism by the glycolytic pathway. This isomerization results in the net production of two molecules of glyceraldehyde 3-phosphate from the cleavage products of fructose 1,6-bisphosphate. [Note: DHAP is utilized in triacylglycerol synthesis (see p. 188).]

F. Oxidation of glyceraldehyde 3-phosphate

The conversion of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate (1,3-BPG) by glyceraldehyde 3-phosphate dehydrogenase is the first oxidation-reduction reaction of glycolysis (Figure 8.18). [Note: Because there is only a limited amount of NAD^+ in the cell, the NADH formed by this reaction must be reoxidized to NAD^+ for glycolysis to continue. Two major mechanisms for oxidizing NADH are 1) the NADH-linked conversion of pyruvate to lactate (anaerobic; see p. 96) and 2) oxidation of NADH via the respiratory chain (aerobic; see p. 74). The latter requires the malate-aspartate and glycerol 3-phosphate substrate shuttles (see p. 79.]

1. **Synthesis of 1,3-bisphosphoglycerate:** The oxidation of the aldehyde group of glyceraldehyde 3-phosphate to a carboxyl group is coupled to the attachment of P_i to the carboxyl group. The high-energy phosphate group at carbon 1 of 1,3-BPG conserves much of the free energy produced by the oxidation of glyceraldehyde 3-phosphate. The energy of this high-energy phosphate drives the synthesis of ATP in the next reaction of glycolysis.

2. **Mechanism of arsenic poisoning:** The toxicity of arsenic is due primarily to the inhibition by trivalent arsenic (arsenite) of enzymes such as the pyruvate dehydrogenase complex, which require lipoic acid as a coenzyme (see p. 110). However, pentavalent arsenic (arsenate) can prevent net ATP and NADH production by glycolysis without inhibiting the pathway itself. It does so by competing with P_i as a substrate for glyceraldehyde 3-phosphate dehydrogenase, forming a complex that spontaneously hydrolyzes to form 3-phosphoglycerate (see Figure 8.18). By bypassing the synthesis of and phosphate transfer from 1,3-BPG, the cell is deprived of energy usually obtained from the glycolytic pathway. [Note: Arsenate also competes with P_i on the F_1 domain of ATP synthase (see p. 77), resulting in formation of ADP-arsenate that is rapidly hydrolyzed.]

3. **Synthesis of 2,3-bisphosphoglycerate in red blood cells:** Some of the 1,3-BPG is converted to 2,3-BPG by the action of bisphosphoglycerate mutase (see Figure 8.18). 2,3-BPG, which is found in only trace amounts in most cells, is present at high concentration in red blood cells (RBCs) and serves to increase O_2 delivery (see p. 31). 2,3-BPG is hydrolyzed by a phosphatase to 3-phosphoglycerate, which is also an intermediate in glycolysis (see Figure 8.18). In the RBC, glycolysis is modified by inclusion of these "shunt" reactions.

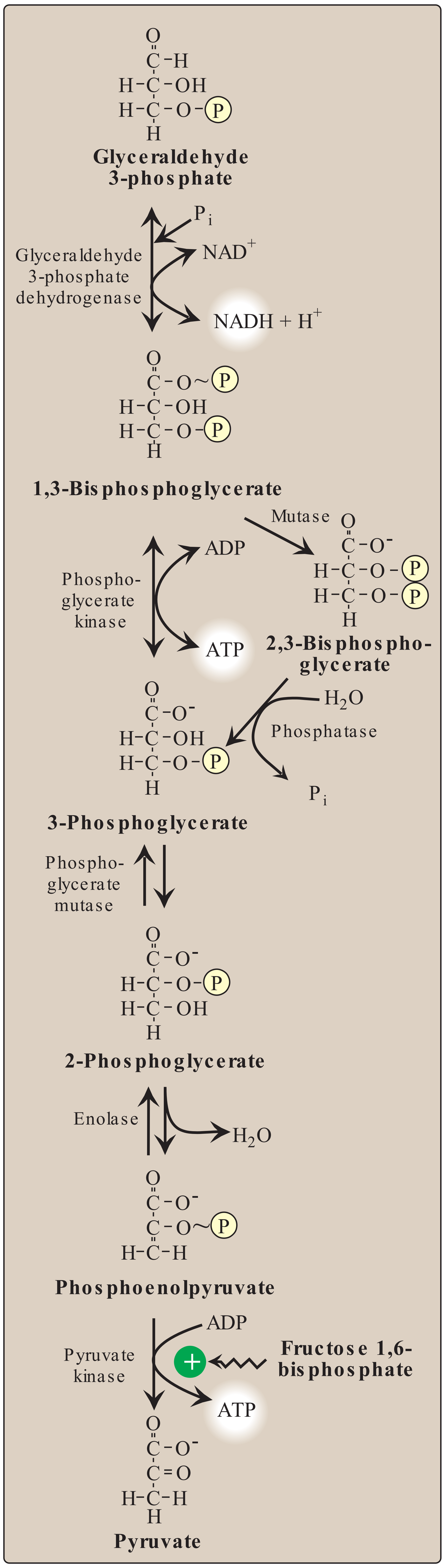

Glyceraldehyde 3-phosphate
P_i
NAD^+
Glyceraldehyde 3-phosphate dehydrogenase
$NADH + H^+$
1,3-Bisphosphoglycerate
Mutase
ADP
Phosphoglycerate kinase
ATP
2,3-Bisphosphoglycerate
H_2O
Phosphatase
P_i
3-Phosphoglycerate
Phosphoglycerate mutase
2-Phosphoglycerate
Enolase
H_2O
Phosphoenolpyruvate
ADP
Pyruvate kinase
+
Fructose 1,6-bisphosphate
ATP
Pyruvate

Figure 8.18
Energy–generating phase: conversion of glyceraldehyde 3-phosphate to pyruvate. NAD(H) = nicotinamide adenine dinucleotide P = phosphate; P_i = inorganic phosphate.

G. Synthesis of 3-phosphoglycerate, producing ATP

When 1,3-BPG is converted to 3-phosphoglycerate, the high-energy phosphate group of 1,3-BPG is used to synthesize ATP from ADP (see Figure 8.18). This reaction is catalyzed by phosphoglycerate kinase, which, unlike most other kinases, is physiologically reversible. Because two molecules of 1,3-BPG are formed from each glucose molecule, this kinase reaction replaces the two ATP molecules consumed by the earlier formation of glucose 6-phosphate and fructose 1,6-bisphosphate. [Note: This is an example of substrate-level phosphorylation, in which the energy needed for the production of a high-energy phosphate comes from a substrate rather than from the electron transport chain (see J. below and p. 113 for other examples).]

H. Shift of the phosphate group

The shift of the phosphate group from carbon 3 to carbon 2 of phosphoglycerate by phosphoglycerate mutase is freely reversible (see Figure 8.18).

I. Dehydration of 2-phosphoglycerate

The dehydration of 2-phosphoglycerate by enolase redistributes the energy within the substrate, resulting in the formation of phosphoenolpyruvate (PEP), which contains a high-energy enol phosphate (see Figure 8.18). The reaction is reversible despite the high-energy nature of the product. [Note: Fluoride inhibits enolase, and water fluoridation reduces lactate production by mouth bacteria, decreasing dental caries.]

J. Formation of pyruvate, producing ATP

The conversion of PEP to pyruvate is catalyzed by pyruvate kinase (PK), the third irreversible reaction of glycolysis. The high-energy enol phosphate in PEP is used to synthesize ATP from ADP and is another example of substrate-level phosphorylation (see Figure 8.18).

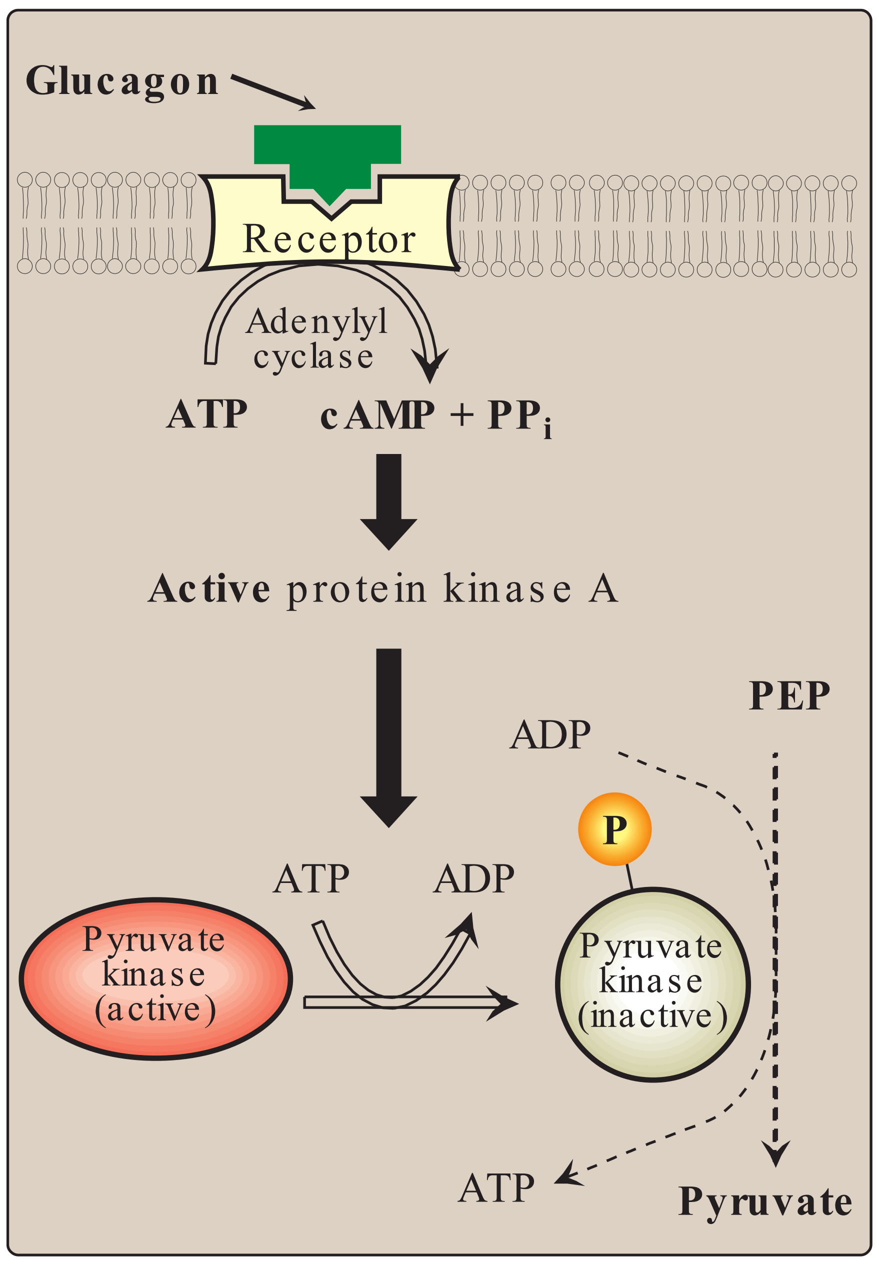


Figure 8.19
Covalent modification of hepatic pyruvate kinase results in inactivation of the enzyme. cAMP = cyclic AMP; PEP = phosphoenolpyruvate; P = phosphate; PP$_i$ = pyrophosphate.

1. **Feedforward regulation:** PK is activated by fructose 1,6-bisphosphate, the product of the phosphofructokinase-1 reaction. This feedforward (instead of the more usual feedback) regulation has the effect of linking the two kinase activities: increased phosphofructokinase activity results in elevated levels of fructose 1,6-bisphosphate, which activates PK.

2. **Covalent modulation of pyruvate kinase:** Phosphorylation by a cAMP-dependent protein kinase leads to inactivation of the hepatic isozyme of PK (Figure 8.19). When blood glucose levels are low, elevated glucagon increases the intracellular level of cAMP, which causes the phosphorylation and inactivation of PK in the liver only. Therefore, PEP is unable to continue in glycolysis and, instead, enters the gluconeogenesis pathway. This, in part, explains the observed inhibition of hepatic glycolysis and stimulation of gluconeogenesis by glucagon. Dephosphorylation of PK by a phosphatase results in reactivation of the enzyme.

3. **Pyruvate kinase deficiency:** Mature RBCs lack mitochondria and are, therefore, completely dependent on glycolysis for ATP production. ATP is required to meet the metabolic needs of RBCs and to fuel the ion pumps necessary for the maintenance of the flexible, biconcave shape that allows them to squeeze through narrow capil-

laries. The anemia observed in glycolytic enzyme deficiencies is a consequence of the reduced rate of glycolysis, leading to decreased ATP production. The resulting alterations in the RBC membrane lead to changes in cell shape and, ultimately, to phagocytosis by cells of the reticuloendothelial system, particularly macrophages of the spleen. The premature death and lysis of RBCs result in hemolytic anemia. Among patients exhibiting the rare genetic defects of glycolytic enzymes, the majority has a deficiency in PK. The effects of PK deficiency are restricted to RBCs and include mild-to-severe nonspherocytic hemolytic anemia, with the severe form requiring regular transfusions. [Note: Hepatic PK is encoded by the same gene as the RBC isozyme. Liver cells show no effect, however, because they have mitochondria and can generate ATP by oxidative phosphorylation.] Severity depends both on the degree of enzyme deficiency (generally 5–35% of normal levels) and on the extent to which RBCs compensate by synthesizing increased levels of 2,3-BPG (see p. 31). Almost all individuals with PK deficiency have a mutant enzyme that shows abnormal properties such as altered kinetics (Figure 8.20). Individuals heterozygous for PK deficiency have resistance to the most severe forms of malaria.

> The tissue-specific expression of PK in RBCs and the liver is the result of differential promoter utilization in transcription (see p. 422) of the gene that encodes both isozymes.

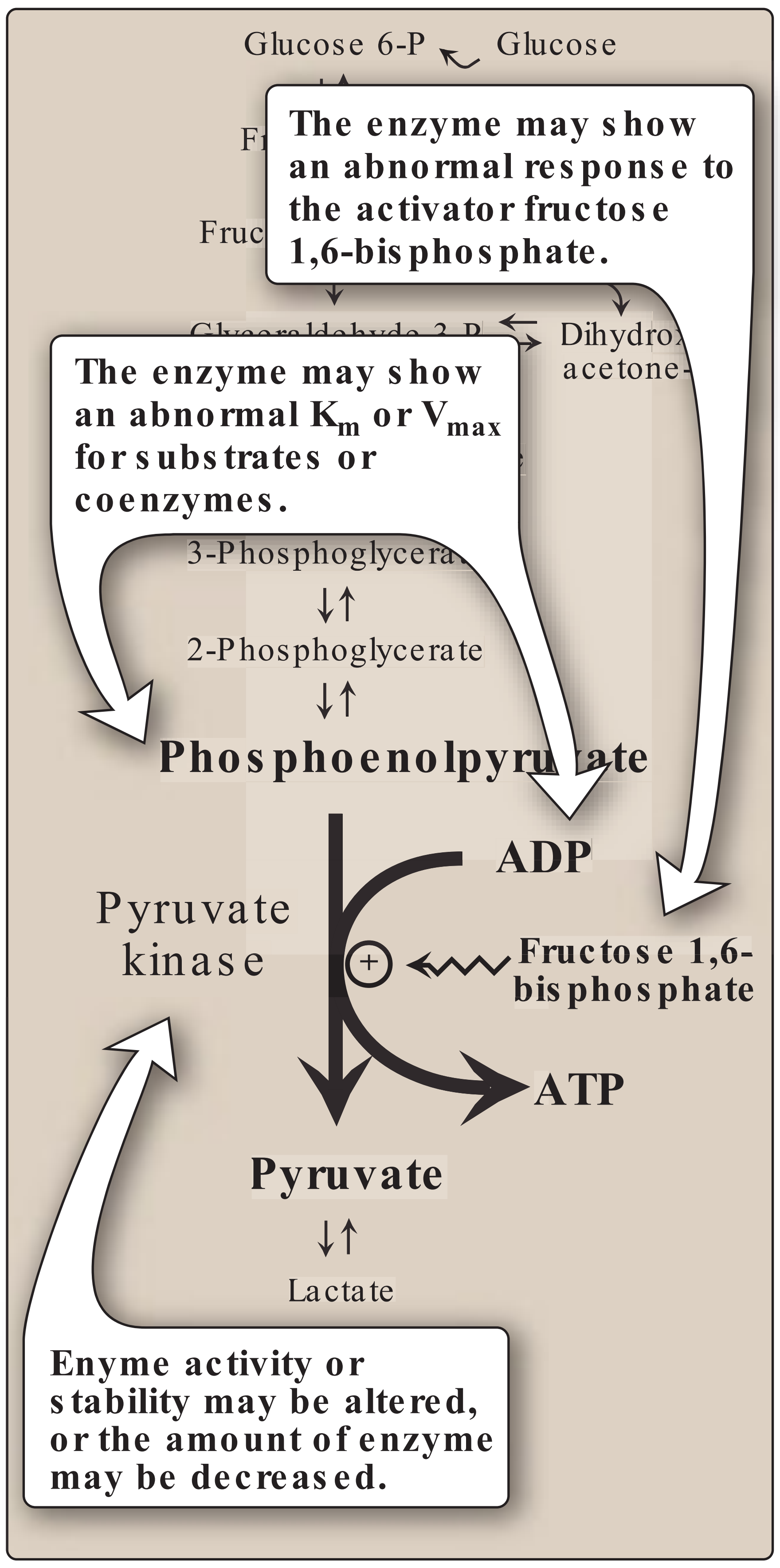

Glucose 6-P
Glucose
The enzyme may show an abnormal response to the activator fructose 1,6-bisphosphate.
Dihydrox acetone-
The enzyme may show an abnormal K_m or V_{max} for substrates or coenzymes.
3-Phosphoglycerate
2-Phosphoglycerate
Phosphoenolpyruvate
ADP
Pyruvate kinase
Fructose 1,6-bisphosphate
ATP
Pyruvate
Lactate
Enyme activity or stability may be altered, or the amount of enzyme may be decreased.

Figure 8.20
Alterations observed with various mutant forms of pyruvate kinase. K_m = Michaelis constant; V_{max} = maximal velocity.

K. Reduction of pyruvate to lactate

Lactate, formed by the action of lactate dehydrogenase, is the final product of anaerobic glycolysis in eukaryotic cells (Figure 8.21). The formation of lactate is the major fate for pyruvate in the lens and cornea of the eye, kidney medulla, testes, leukocytes, and RBCs, because these are all poorly vascularized and/or lack mitochondria.

1. **Lactate formation in muscle:** In exercising skeletal muscle, NADH production (by glyceraldehyde 3-phosphate dehydrogenase and by the three NAD^+-linked dehydrogenases of the TCA cycle; see p. 112) exceeds the oxidative capacity of the respiratory chain. This results in an elevated $NADH/NAD^+$ ratio, favoring reduction of pyruvate to lactate. Therefore, during intense exercise, lactate accumulates in muscle, causing a drop in the intracellular pH, potentially resulting in cramps. Much of this lactate eventually diffuses into the bloodstream and can be used by the liver to make glucose (see p. 118).

2. **Lactate utilization:** The direction of the lactate dehydrogenase reaction depends on the relative intracellular concentrations of pyruvate and lactate and on the ratio of $NADH/NAD^+$ in the cell. For example, in the liver and heart, the ratio of $NADH/NAD^+$ is lower than in exercising muscle. These tissues oxidize lactate (obtained from the blood) to pyruvate. In the liver, pyruvate is either converted to glucose by gluconeogenesis or oxidized in the TCA cycle. Heart muscle exclusively oxidizes lactate to CO_2 and H_2O via the TCA cycle.

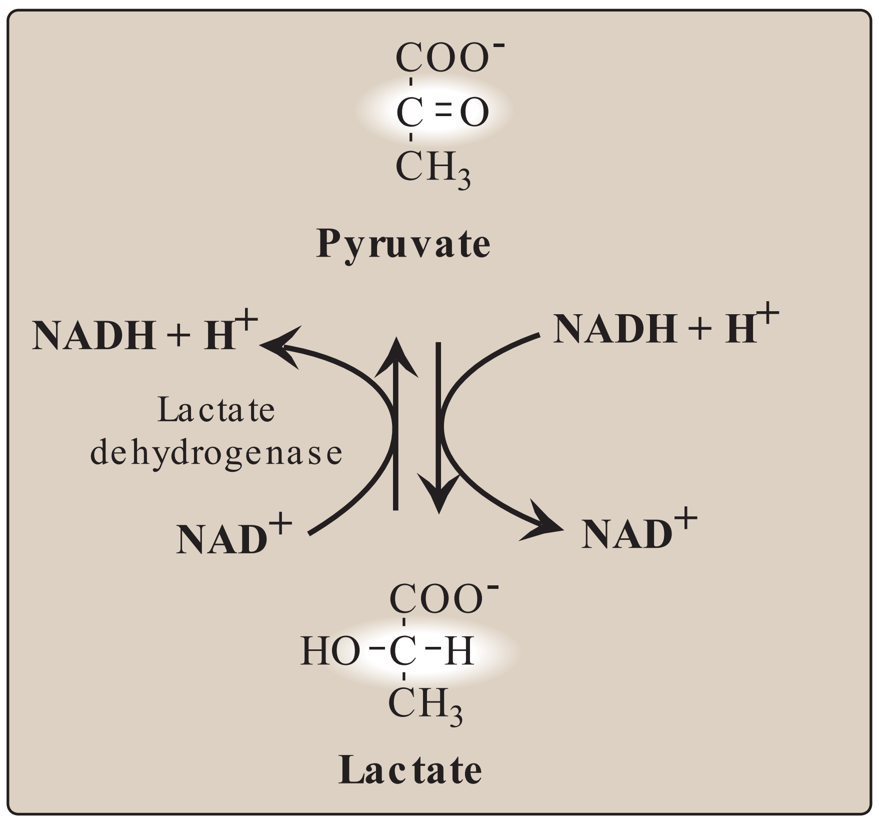

COO^-
C=O
CH_3
Pyruvate
$NADH + H^+$
$NADH + H^+$
Lactate dehydrogenase
NAD^+
NAD^+
COO^-
HO-C-H
CH_3
Lactate

Figure 8.21
Interconversion of pyruvate and lactate. [Note: Lactate produced in muscle enters the circulation, is picked up by liver through facilitated diffusion, and is oxidized to pyruvate. Pyruvate is used by liver to make glucose.] NAD(H) = nicotinamide adenine dinucleotide.

3. **Lactic acidosis:** Elevated concentrations of lactate in the plasma, termed lactic acidosis (a type of metabolic acidosis), occur when there is a collapse of the circulatory system, such as in myocardial infarction, pulmonary embolism, and uncontrolled hemorrhage, or when an individual is in shock. The failure to bring adequate amounts of oxygen to the tissues results in impaired oxidative phosphorylation and decreased ATP synthesis. To survive, the cells rely on anaerobic glycolysis for generating ATP, producing lactic acid as the end product. [Note: Production of even meager amounts of ATP may be life-saving during the period required to reestablish adequate blood flow to the tissues.] The excess oxygen required to recover from a period when the availability of oxygen has been inadequate is termed the "oxygen debt."

> The oxygen debt is often related to patient morbidity or mortality. In many clinical situations, measuring the blood levels of lactic acid allows the rapid, early detection of oxygen debt in patients and the monitoring of their recovery.

L. Energy yield from glycolysis

Despite the production of some ATP during glycolysis, the end product, pyruvate or lactate, still contains most of the energy originally contained in glucose. The TCA cycle is required to release that energy completely (see p. 109).

1. **Anaerobic glycolysis:** Two molecules of ATP are generated for each molecule of glucose converted to two molecules of lactate (Figure 8.22). There is no net production or consumption of NADH.

2. **Aerobic glycolysis:** The direct consumption and formation of ATP is the same as in anaerobic glycolysis (that is, a net gain of two ATP per molecule of glucose). Two molecules of NADH are also produced per molecule of glucose. Ongoing aerobic glycolysis requires the oxidation of most of this NADH by the electron transport chain, producing approximately three ATP for each NADH molecule entering the chain (see p. 77). [Note: NADH cannot cross the inner mitochondrial membrane, and substrate shuttles are required (see p. 79).]

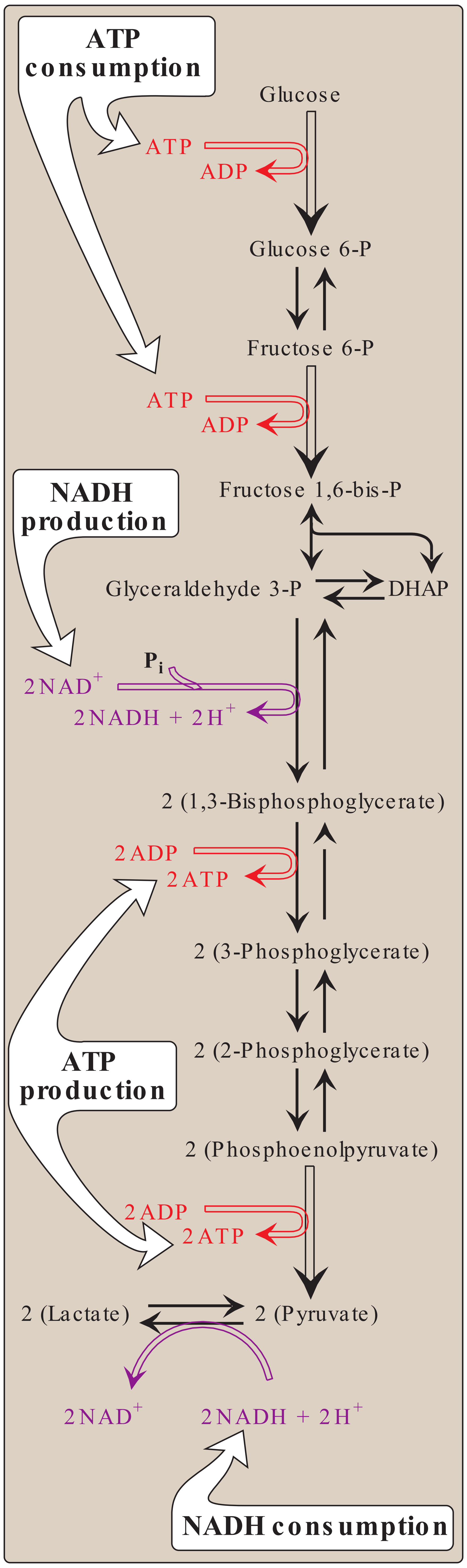


Figure 8.22
Summary of anaerobic glycolysis. Reactions involving the production or consumption of ATP or NADH are indicated. The three irreversible reactions of glycolysis are shown with thick arrows. DHAP = dihydroxyacetone phosphate; NAD(H) = nicotinamide adenine dinucleotide; P = phosphate.

VI. HORMONAL REGULATION OF GLYCOLYSIS

The regulation of glycolysis by allosteric activation or inhibition, or the covalent phosphorylation/dephosphorylation of rate-limiting enzymes, is short-term (that is, they influence glucose consumption over periods of minutes or hours). Superimposed on these moment-to-moment effects are slower, and often more profound, hormonal influences on gene expression, or the amount of enzyme protein synthesized. These effects can result in 10-fold to 20-fold increases in enzyme activity that typically occur over hours to days. Although the current focus is on glycolysis, reciprocal changes occur in the rate-limiting enzymes of gluconeogenesis, which are described in Chapter 10 (see p. 117). Regular consumption of meals rich in carbohydrate or administration of insulin initiates an increase in the amount of glucokinase, phosphofructokinase, and PK in the liver (Figure 8.23). These changes reflect an increase in gene transcription, resulting in increased enzyme synthesis. High activity of these three enzymes favors the conversion of glucose to pyruvate, a characteristic of the absorptive state (see p. 321). Conversely, gene transcription and synthesis of glucokinase, phosphofructokinase, and PK are decreased when plasma glucagon is high and insulin is low (for example, as seen in fasting or diabetes).

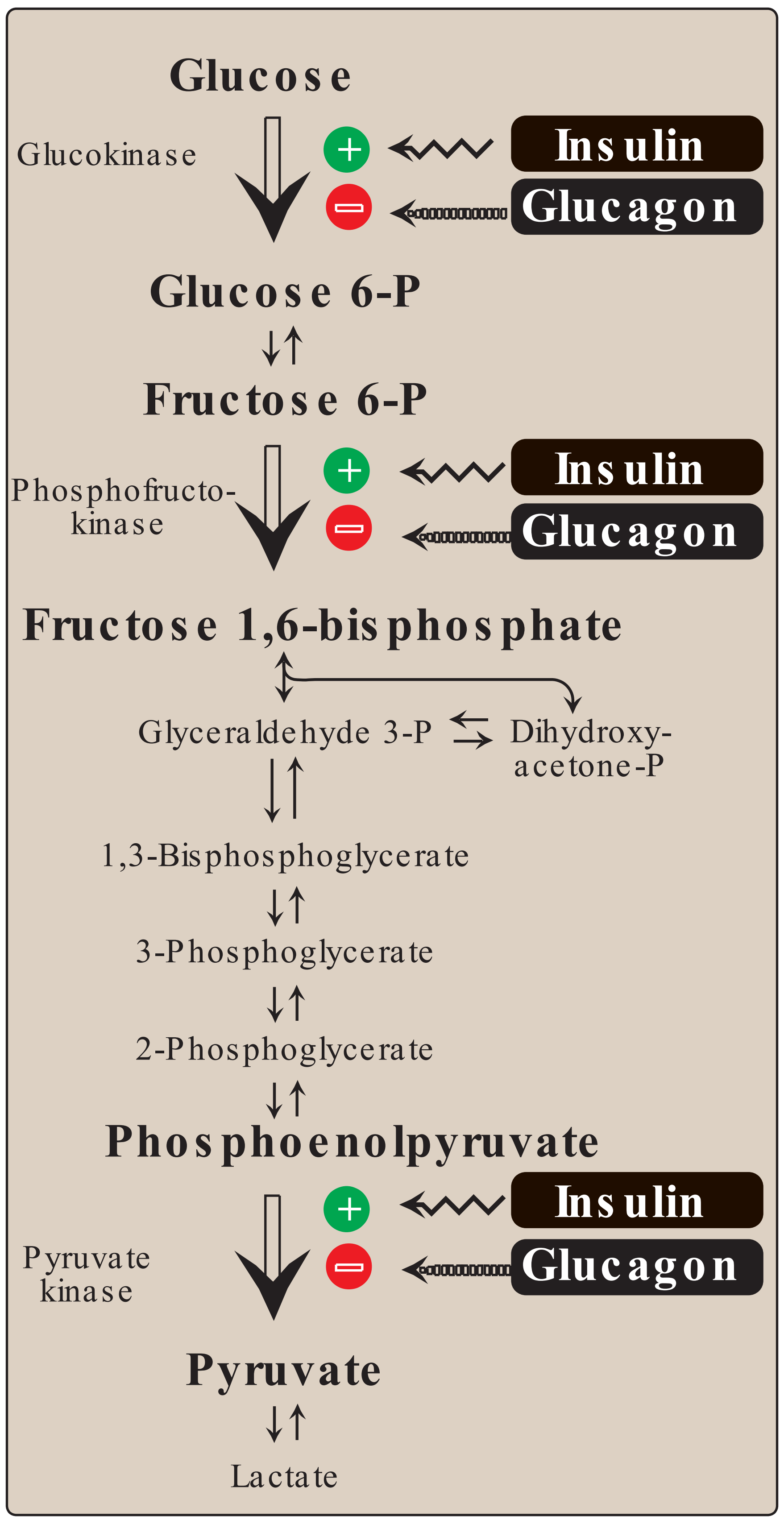


Figure 8.23
Effect of insulin and glucagon on the synthesis of key enzymes of glycolysis in liver. P = phosphate.

VII. ALTERNATE FATES OF PYRUVATE

A. Oxidative decarboxylation of pyruvate

Oxidative decarboxylation of pyruvate by the pyruvate dehydrogenase complex is an important pathway in tissues with a high oxidative capacity such as cardiac muscle (Figure 8.24). Pyruvate dehydrogenase irreversibly converts pyruvate, the end product of glycolysis, into acetyl CoA, a major fuel for the TCA cycle (see p. 109) and the building block for fatty acid synthesis (see p. 183).

B. Carboxylation of pyruvate to oxaloacetate

Carboxylation of pyruvate to oxaloacetate by pyruvate carboxylase is a biotin-dependent reaction (see Figure 8.24). This reaction is important because it replenishes the TCA cycle intermediates and provides substrate for gluconeogenesis (see p. 118).

C. Reduction of pyruvate to ethanol (microorganisms)

The conversion of pyruvate to ethanol occurs by the two reactions summarized in Figure 8.24. The decarboxylation of pyruvate by pyruvate decarboxylase occurs in yeast and certain other microorganisms but not in humans. The enzyme requires thiamine pyrophosphate as a coenzyme and catalyzes a reaction similar to that described for pyruvate dehydrogenase (see p. 110).

VIII. CHAPTER SUMMARY

Most pathways can be classified as either **catabolic** (**degrade** complex molecules to a few simple products) or **anabolic** (**synthesize** complex end products from simple precursors). **Catabolic reactions** also **capture chemical energy** in the form of **ATP** from the degradation of energy-rich molecules. **Anabolic reactions require energy**, which is generally provided by the hydrolysis of ATP. The rate of a metabolic pathway can respond to **regulatory signals** such as **allosteric activators** or **inhibitors** that arise from **within the cell**. Signaling **between cells** provides for the integration of metabolism. The most important route of this communication is **chemical signaling** (for example, by **hormones** or **neurotransmitters**). **Second messenger molecules** transduce a chemical signal (hormone or neurotransmitter) to appropriate intracellular responders.

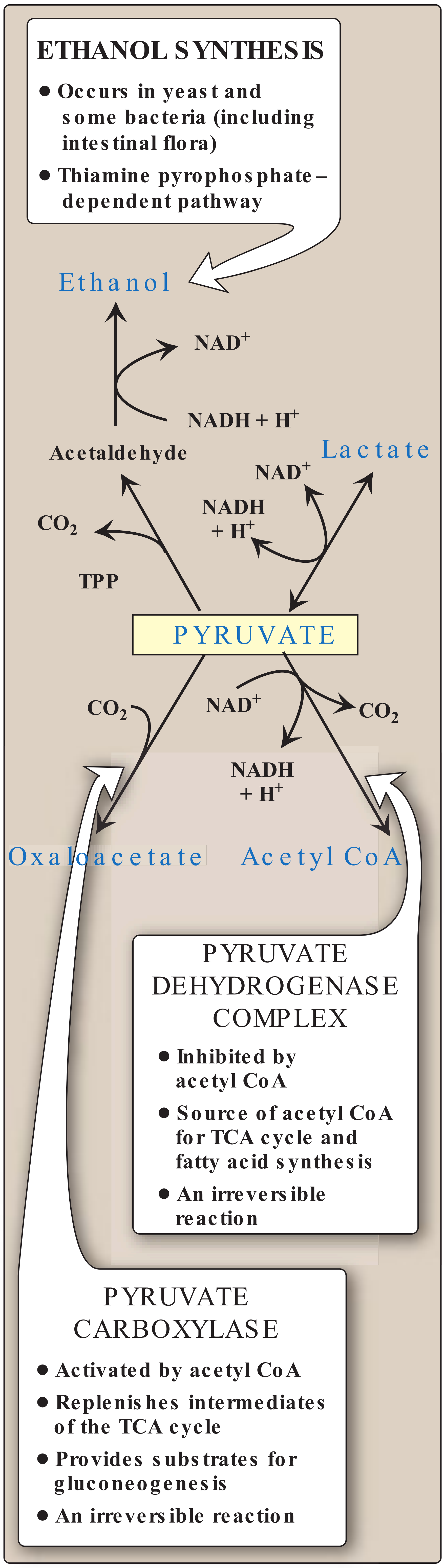

ETHANOL SYNTHESIS
● Occurs in yeast and some bacteria (including intestinal flora)
● Thiamine pyrophosphate–dependent pathway
Ethanol
NAD+
NADH + H+
Acetaldehyde
Lactate
NAD+
NADH + H+
CO2
TPP
PYRUVATE
CO2
NAD+
CO2
NADH + H+
Oxaloacetate
Acetyl CoA
PYRUVATE DEHYDROGENASE COMPLEX
● Inhibited by acetyl CoA
● Source of acetyl CoA for TCA cycle and fatty acid synthesis
● An irreversible reaction
PYRUVATE CARBOXYLASE
● Activated by acetyl CoA
● Replenishes intermediates of the TCA cycle
● Provides substrates for gluconeogenesis
● An irreversible reaction

Figure 8.24
Summary of the metabolic fates of pyruvate. TPP = thiamine pyrophosphate. TCA = tricarboxylic acid; NAD(H) = nicotinamide adenine dinucleotide; CoA = coenzyme A.

Adenylyl cyclase is a cell membrane enzyme that synthesizes **cyclic AMP (cAMP)** in response to chemical signals, such as the hormones **glucagon** and **epinephrine**. Following binding of a hormone to its **cell-surface receptor**, a GTP-dependent regulatory protein (**G protein**) is activated that, in turn, activates adenylyl cyclase. The cAMP produced activates a protein kinase, which phosphorylates a cadre of enzymes, causing their activation or deactivation. Phosphorylation is reversed by protein phosphatases. **Aerobic glycolysis**, in which **pyruvate** is the end product, occurs in cells with mitochondria and an adequate supply of oxygen (Figure 8.25). **Anaerobic glycolysis**, in which **lactic acid** is the end product, occurs in cells that lack mitochondria and in cells deprived of sufficient oxygen. Glucose is transported across membranes by one of 14 **glucose transporter isoforms** (GLUTs). **GLUT-1** is abundant in **erythrocytes** and the **brain**, **GLUT-4** (which is **insulin dependent**) is found in **muscle** and **adipose tissue**, and **GLUT-2** is found in the **liver**, **kidney**, and **β cells** of the pancreas. The conversion of glucose to pyruvate (**glycolysis**; Figure 8.25) occurs in two stages: an **energy–investment phase** in which phosphorylated intermediates are synthesized at the expense of ATP, and an **energy-generation phase**, in which ATP is produced. In the energy-investment phase, glucose is phosphorylated by hexokinase (found in **most tissues**) or glucokinase (a hexokinase found in **liver cells** and the **β cells** of the pancreas). Hexokinase has a **high affinity** (**low K_m**) and a **low V_{max}** for glucose and is **inhibited** by **glucose 6-phosphate**. Glucokinase has a **high K_m** and a **high V_{max}** for glucose. It is indirectly **inhibited** by **fructose 6-phosphate** and **activated** by **glucose**. The **transcription** of the gene for glucokinase is **enhanced by insulin**. Glucose 6-phosphate is isomerized to fructose 6-phosphate, which is phosphorylated to **fructose 1,6-bisphosphate** by phosphofructokinase-1 (PFK-1). This enzyme is **allosterically inhibited** by **ATP** and **citrate** and **activated** by **AMP**. **Fructose 2,6-bisphosphate**, whose synthesis by phosphofructokinase-2 (PFK-2) is **activated** by **insulin**, is the most potent allosteric activator of PFK-1. A total of **two ATP are used** during this phase of glycolysis. Fructose 1,6-bisphosphate is cleaved to form two trioses that are further metabolized by the glycolytic pathway, forming pyruvate. During these reactions, **four ATP** and **two NADH are produced** from ADP and NAD$^+$. The final step in pyruvate synthesis from phosphoenolpyruvate is catalyzed by pyruvate kinase (PK). This enzyme is **allosterically activated** by **fructose 1,6-bisphosphate** and **hormonally activated** by **insulin** and **inhibited** in the liver by **glucagon** via the **cAMP pathway**. PK **deficiency** accounts for the majority of all inherited defects in glycolytic enzymes. Effects are restricted to **erythrocytes** and present as mild to severe **chronic, nonspherocytic hemolytic anemia**. In **anaerobic glycolysis**, NADH is reoxidized to NAD$^+$ by the **conversion of pyruvate to lactate**. This occurs in cells, such as **erythrocytes**, that have few or no mitochondria, and in tissues, such as **exercising muscle**, where production of NADH exceeds the oxidative capacity of the respiratory chain. Elevated concentrations of lactate in the plasma (**lactic acidosis**) occur when there is a **collapse of the circulatory system** or when an individual is in **shock**. Pyruvate can be 1) **oxidatively decarboxylated** by pyruvate dehydrogenase, producing **acetyl coenzyme A**; 2) **carboxylated** to **oxaloacetate** (a tricarboxylic acid cycle intermediate) by pyruvate carboxylase; or 3) **reduced** by microorganisms to **ethanol** by pyruvate decarboxylase.

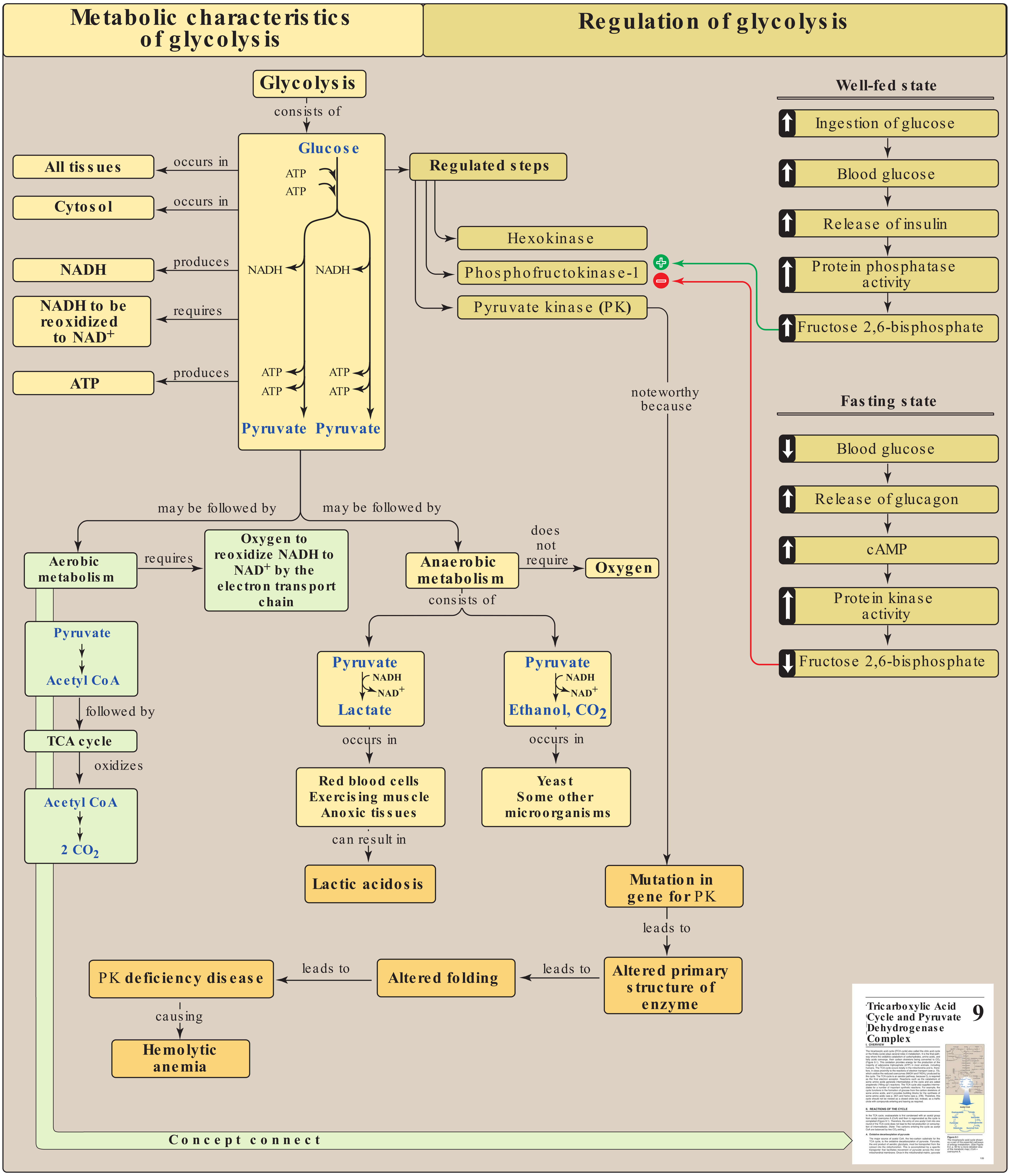

Metabolic characteristics of glycolysis
Regulation of glycolysis
Glycolysis
consists of
Glucose
ATP
ATP
NADH
NADH
ATP
ATP
ATP
ATP
Pyruvate
Pyruvate
occurs in
All tissues
occurs in
Cytosol
produces
NADH
requires
NADH to be reoxidized to NAD$^+$
produces
ATP
Regulated steps
Hexokinase
Phosphofructokinase-1
Pyruvate kinase (PK)
Well-fed state
↑ Ingestion of glucose
↑ Blood glucose
↑ Release of insulin
↑ Protein phosphatase activity
↑ Fructose 2,6-bisphosphate
+
−
noteworthy because
Fasting state
↓ Blood glucose
↑ Release of glucagon
↑ cAMP
↑ Protein kinase activity
↓ Fructose 2,6-bisphosphate
may be followed by
may be followed by
Aerobic metabolism
requires
Oxygen to reoxidize NADH to NAD$^+$ by the electron transport chain
Anaerobic metabolism
does not require
Oxygen
consists of
Pyruvate
Acetyl CoA
followed by
TCA cycle
oxidizes
Acetyl CoA
2 CO$_2$
Pyruvate
NADH
NAD$^+$
Lactate
Pyruvate
NADH
NAD$^+$
Ethanol, CO$_2$
occurs in
occurs in
Red blood cells
Exercising muscle
Anoxic tissues
Yeast
Some other microorganisms
can result in
Lactic acidosis
Mutation in gene for PK
leads to
Altered primary structure of enzyme
leads to
Altered folding
leads to
PK deficiency disease
causing
Hemolytic anemia
Concept connect
Tricarboxylic Acid Cycle and Pyruvate Dehydrogenase Complex
9

Figure 8.25
Key concept map for glycolysis. NAD(H) = nicotinamide adenine dinucleotide; cAMP = cyclic adenosine monophosphate; CoA = coenzyme A; TCA = tricarboxylic acid.

Tricarboxylic Acid Cycle and Pyruvate Dehydrogenase Complex

9

I. OVERVIEW

The tricarboxylic acid cycle ([TCA cycle] also called the citric acid cycle or the Krebs cycle) plays several roles in metabolism. It is the final pathway where the oxidative catabolism of carbohydrates, amino acids, and fatty acids converge, their carbon skeletons being converted to CO_2 (Figure 9.1). This oxidation provides energy for the production of the majority of adenosine triphosphate (ATP) in most animals, including humans. The TCA cycle occurs totally in the mitochondria and is, therefore, in close proximity to the reactions of electron transport (see p. 73), which oxidize the reduced coenzymes (NADH and $FADH_2$) produced by the cycle. The TCA cycle is an aerobic pathway, because O_2 is required as the final electron acceptor. Reactions such as the catabolism of some amino acids generate intermediates of the cycle and are called anaplerotic ("filling up") reactions. The TCA cycle also supplies intermediates for a number of important synthetic reactions. For example, the cycle functions in the formation of glucose from the carbon skeletons of some amino acids, and it provides building blocks for the synthesis of some amino acids (see p. 267) and heme (see p. 278). Therefore, this cycle should not be viewed as a closed circle but, instead, as a traffic circle with compounds entering and leaving as required.

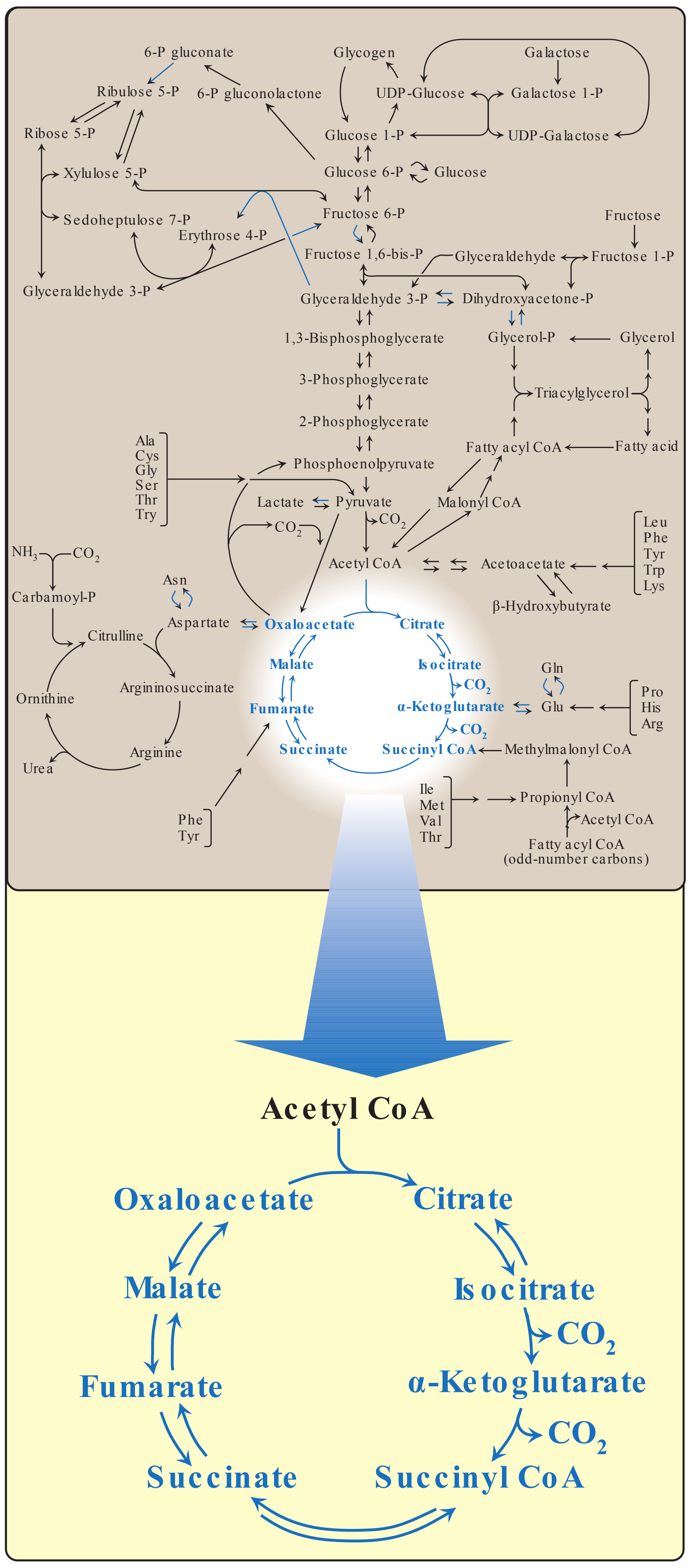

6-P gluconate
Glycogen
Galactose
Ribulose 5-P
6-P gluconolactone
UDP-Glucose
Galactose 1-P
Ribose 5-P
Glucose 1-P
UDP-Galactose
Xylulose 5-P
Glucose 6-P
Glucose
Fructose 6-P
Fructose
Sedoheptulose 7-P
Erythrose 4-P
Fructose 1,6-bis-P
Glyceraldehyde
Fructose 1-P
Glyceraldehyde 3-P
Glyceraldehyde 3-P
Dihydroxyacetone-P
1,3-Bisphosphoglycerate
Glycerol-P
Glycerol
3-Phosphoglycerate
Triacylglycerol
2-Phosphoglycerate
Fatty acyl CoA
Fatty acid
Ala
Cys
Gly
Ser
Thr
Try
Phosphoenolpyruvate
Lactate
Pyruvate
Malonyl CoA
CO2
CO2
NH3
CO2
Acetyl CoA
Acetoacetate
Leu
Phe
Tyr
Trp
Lys
Carbamoyl-P
Asn
β-Hydroxybutyrate
Citrulline
Aspartate
Oxaloacetate
Citrate
Malate
Isocitrate
Gln
CO2
Ornithine
Argininosuccinate
Fumarate
α-Ketoglutarate
Glu
Pro
His
Arg
CO2
Succinate
Succinyl CoA
Methylmalonyl CoA
Urea
Arginine
Ile
Met
Val
Thr
Propionyl CoA
Phe
Tyr
Acetyl CoA
Fatty acyl CoA
(odd-number carbons)
Acetyl CoA
Oxaloacetate
Citrate
Malate
Isocitrate
CO2
Fumarate
α-Ketoglutarate
CO2
Succinate
Succinyl CoA

Figure 9.1
The tricarboxylic acid cycle shown as a part of the essential pathways of energy metabolism. (See Figure 8.2, p. 92 for a more detailed view of the metabolic map.) CoA = coenzyme A.

II. REACTIONS OF THE CYCLE

In the TCA cycle, oxaloacetate is first condensed with an acetyl group from acetyl coenzyme A (CoA) and then is regenerated as the cycle is completed (Figure 9.1). Therefore, the entry of one acetyl CoA into one round of the TCA cycle does not lead to the net production or consumption of intermediates. [Note: Two carbons entering the cycle as acetyl CoA are balanced by two CO_2 exiting.]

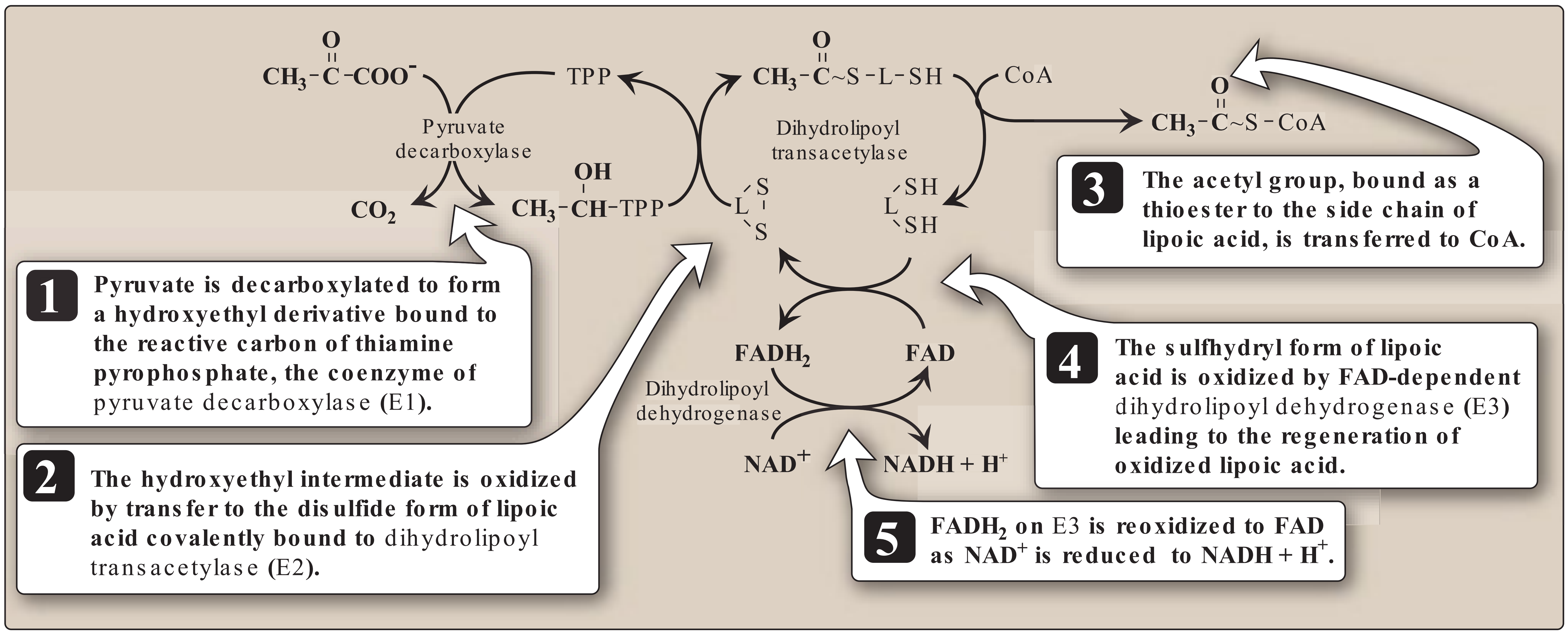
A. Oxidative decarboxylation of pyruvate

The major source of acetyl CoA, the two-carbon substrate for the TCA cycle, is the oxidative decarboxylation of pyruvate. Pyruvate, the end product of aerobic glycolysis, must be transported from the cytosol into the mitochondrion. This is accomplished by a specific transporter that facilitates movement of pyruvate across the inner mitochondrial membrane. Once in the mitochondrial matrix, pyruvate is converted to acetyl CoA by the pyruvate dehydrogenase complex (PDH complex), which is a multienzyme complex. [Note: Strictly speaking, the PDH complex is not part of the TCA cycle, but it supplies substrate for the cycle.]

1. **Component enzymes:** The PDH complex is a protein aggregate of multiple copies of three enzymes, pyruvate carboxylase (E1, sometimes called pyruvate dehydrogenase), dihydrolipoyl transacetylase (E2), and dihydrolipoyl dehydrogenase (E3). Each catalyzes a part of the overall reaction (Figure 9.2). Their physical association links the reactions in proper sequence without the release of intermediates. In addition to the enzymes participating in the conversion of pyruvate to acetyl CoA, the complex also contains two tightly bound regulatory enzymes, pyruvate dehydrogenase kinase (PDH kinase) and pyruvate dehydrogenase phosphatase (PDH phosphatase).

2. **Coenzymes:** The PDH complex contains five coenzymes that act as carriers or oxidants for the intermediates of the reactions shown in Figure 9.2. E1 requires thiamine pyrophosphate (TPP), E2 requires lipoic acid and CoA, and E3 requires flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NAD^+). [Note: TPP, lipoic acid, and FAD are tightly bound to the enzymes and function as coenzymes-prosthetic groups (see p. 54).]

> Deficiencies of thiamine or niacin can cause serious central nervous system problems. This is because brain cells are unable to produce sufficient ATP (via the TCA cycle) if the PDH complex is inactive. Wernicke-Korsakoff, an encephalopathy-psychosis syndrome due to thiamine deficiency, may be seen with alcohol abuse.



1 **Pyruvate is decarboxylated to form a hydroxyethyl derivative bound to the reactive carbon of thiamine pyrophosphate, the coenzyme of** pyruvate decarboxylase **(**E1**).**

2 **The hydroxyethyl intermediate is oxidized by transfer to the disulfide form of lipoic acid covalently bound to** dihydrolipoyl transacetylase **(**E2**).**

3 **The acetyl group, bound as a thioester to the side chain of lipoic acid, is transferred to CoA.**

4 **The sulfhydryl form of lipoic acid is oxidized by FAD-dependent** dihydrolipoyl dehydrogenase **(**E3**) leading to the regeneration of oxidized lipoic acid.**

5 **$FADH_2$ on** E3 **is reoxidized to FAD as NAD^+ is reduced to NADH + H^+.**

Figure 9.2

Mechanism of action of the pyruvate dehydrogenase complex. [Note: All the coenzymes of the complex, except for lipoic acid, are derived from vitamins. TPP is from thiamine, FAD from riboflavin, NAD from niacin, and CoA from pantothenic acid.] TPP = thiamine pyrophosphate; L = lipoic acid; CoA = coenzyme A; FAD(H_2) = flavin adenine dinucleotide; NAD(H) = nicotinamide adenine dinucleotide.

3. **Regulation of the pyruvate dehydrogenase complex:** Covalent modifications by the two regulatory enzymes that are part of the complex alternately activate and inactivate E1. The cyclic AMP–independent PDH kinase phosphorylates and, thereby, inactivates E1, whereas PDH phosphatase dephosphorylates and activates E1 (Figure 9.3). The kinase itself is allosterically activated by ATP, acetyl CoA, and NADH. Therefore, in the presence of these high-energy signals, the PDH complex is turned off. [Note: It is actually the rise in the ATP/ADP, NADH/NAD$^+$, or acetyl CoA/CoA ratios that affects enzymic activity.] Pyruvate is a potent inhibitor of PDH kinase. Thus, if pyruvate concentrations are elevated, E1 will be maximally active. Calcium (Ca^{2+}) is a strong activator of PDH phosphatase, stimulating E1 activity. This is particularly important in skeletal muscle, where release of Ca^{2+} during contraction stimulates the PDH complex and, thereby, energy production. [Note: Although covalent regulation by the kinase and phosphatase is primary, the complex is also subject to product (NADH and acetyl CoA) inhibition.]

4. **Pyruvate dehydrogenase complex deficiency:** A deficiency in the activity of the α subunit of the dimeric E1 component of the PDH complex, although rare, is the most common biochemical cause of congenital lactic acidosis. This enzyme deficiency results in an inability to convert pyruvate to acetyl CoA, causing pyruvate to be shunted to lactate via lactate dehydrogenase (see p. 103). This creates particular problems for the brain, which relies on the TCA cycle for most of its energy and is particularly sensitive to acidosis. Symptoms are variable and include neurodegeneration; muscle spasticity; and, in the neonatal onset form, early death. The gene for the α subunit is X linked, and, because both males and females may be affected, the deficiency is classified as X-linked dominant. Although there is no proven treatment for PDH complex deficiency, dietary restriction of carbohydrate and supplementation with thiamine may reduce symptoms in select patients.

Leigh syndrome (subacute necrotizing encephalomyelopathy) is a rare, progressive, neurodegenerative disorder caused by defects in mitochondrial ATP production, primarily as a result of mutations in genes that code for proteins of the PDH complex, the electron transport chain, or ATP synthase. Both nuclear and mitochondrial DNA can be affected.

5. **Mechanism of arsenic poisoning:** As previously described (see p. 101), pentavalent arsenic (arsenate) can interfere with glycolysis at the glyceraldehyde 3-phosphate step, thereby decreasing ATP production. "Arsenic poisoning" is, however, due primarily to inhibition of enzymes that require lipoic acid as a coenzyme, including E2 of the PDH complex, α-ketoglutarate dehydrogenase (see below), and branched-chain α-keto acid dehydrogenase (see p. 266). Arsenite (the trivalent form of arsenic) forms a stable complex with the thiol (–SH) groups of lipoic acid, making that compound unavailable to serve as a coenzyme. When it binds to lipoic acid in the PDH complex, pyruvate (and, consequently, lactate)

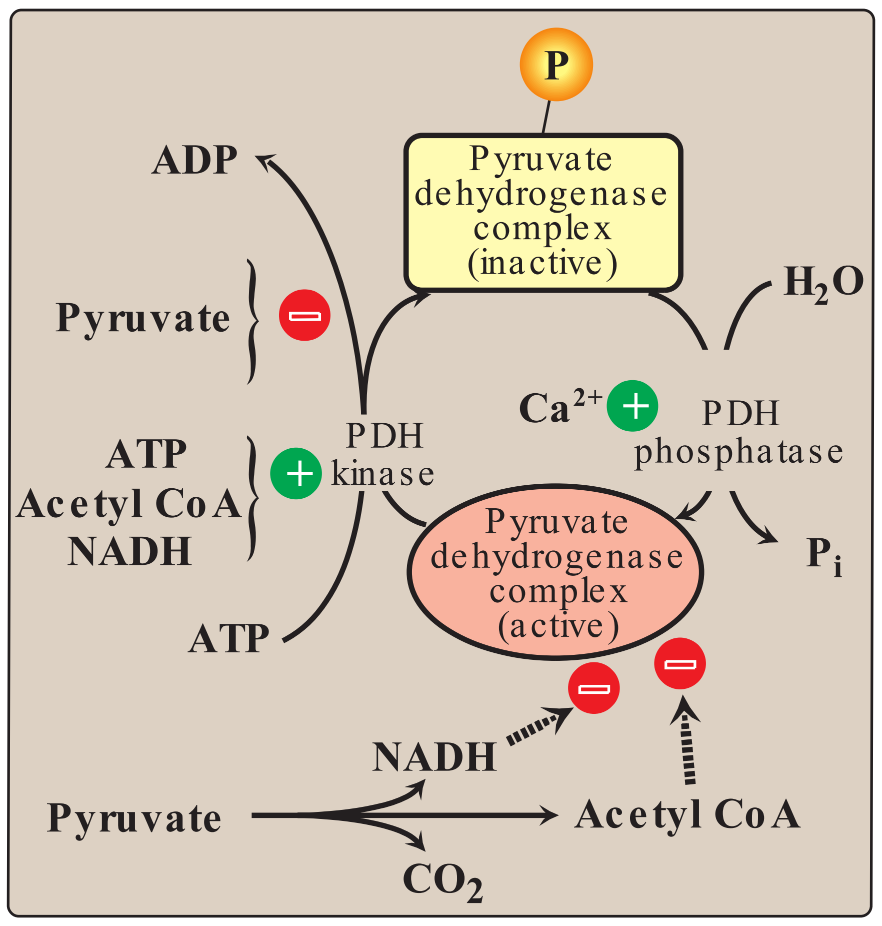


Figure 9.3
Regulation of pyruvate dehydrogenase (PDH) complex. [⇢ denotes product inhibition.]

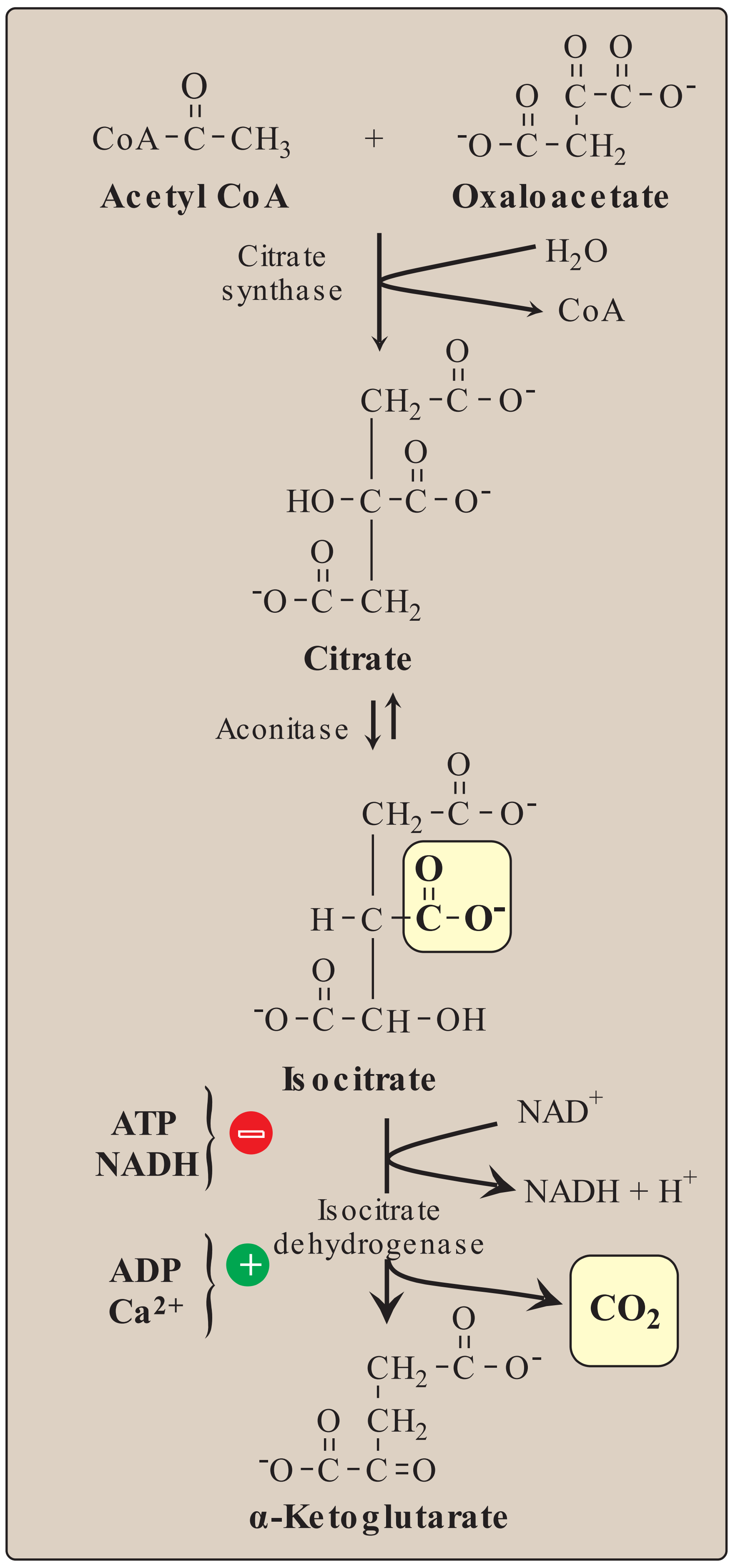


Figure 9.4
Formation of α-ketoglutarate from acetyl coenzyme A (CoA) and oxaloacetate. NAD(H) = nicotinamide adenine dinucleotide

accumulates. As with PDH complex deficiency, this particularly affects the brain, causing neurologic disturbances and death.

B. Synthesis of citrate from acetyl coenzyme A and oxaloacetate

The condensation of acetyl CoA and oxaloacetate (OAA) to form citrate (a tricarboxylic acid) is catalyzed by citrate synthase (Figure 9.4). This aldol condensation has an equilibrium far in the direction of citrate synthesis. In humans, citrate synthase is not an allosteric enzyme. It is inhibited by its product, citrate. Substrate availability is another means of regulation for citrate synthase. The binding of OAA causes a conformational change in the enzyme that generates a binding site for acetyl CoA. [Note: Citrate, in addition to being an intermediate in the TCA cycle, provides a source of acetyl CoA for the cytosolic synthesis of fatty acids (see p. 183). Citrate also inhibits phosphofructokinase-1 (PFK-1), the rate-limiting enzyme of glycolysis (see p. 99), and activates acetyl CoA carboxylase (the rate-limiting enzyme of fatty acid synthesis; see p. 183).]

C. Isomerization of citrate

Citrate is isomerized to isocitrate by aconitase (aconitate hydratase), an Fe-S protein (see Figure 9.4). [Note: Aconitase is inhibited by fluoroacetate, a plant toxin that is used as a pesticide. Fluoroacetate is converted to fluoroacetyl CoA, which condenses with OAA to form fluorocitrate (a potent inhibitor of aconitase), resulting in citrate accumulation.]

D. Oxidative decarboxylation of isocitrate

Isocitrate dehydrogenase catalyzes the irreversible oxidative decarboxylation of isocitrate, yielding the first of three NADH molecules produced by the cycle and the first release of CO_2 (see Figure 9.4). This is one of the rate-limiting steps of the TCA cycle. The enzyme is allosterically activated by ADP (a low-energy signal) and Ca^{2+} and is inhibited by ATP and NADH, levels of which are elevated when the cell has abundant energy stores.

E. Oxidative decarboxylation of α-ketoglutarate

The conversion of α-ketoglutarate to succinyl CoA is catalyzed by the α-ketoglutarate dehydrogenase complex, a protein aggregate of multiple copies of three enzymes (Figure 9.5). The mechanism of this oxidative decarboxylation is very similar to that used for the conversion of pyruvate to acetyl CoA by the PDH complex. The reaction releases the second CO_2 and produces the second NADH of the cycle. The coenzymes required are TPP, lipoic acid, FAD, NAD^+, and CoA. Each functions as part of the catalytic mechanism in a way analogous to that described for the PDH complex (see p. 110). The equilibrium of the reaction is far in the direction of succinyl CoA, a high-energy thioester similar to acetyl CoA. α-Ketoglutarate dehydrogenase complex is inhibited by its products, NADH and succinyl CoA, and activated by Ca^{2+}. However, it is not regulated by phosphorylation/dephosphorylation reactions as described for PDH complex. [Note: α-Ketoglutarate is also produced by the oxidative deamination (see p. 252) and transamination of the amino acid glutamate (see p. 250).]

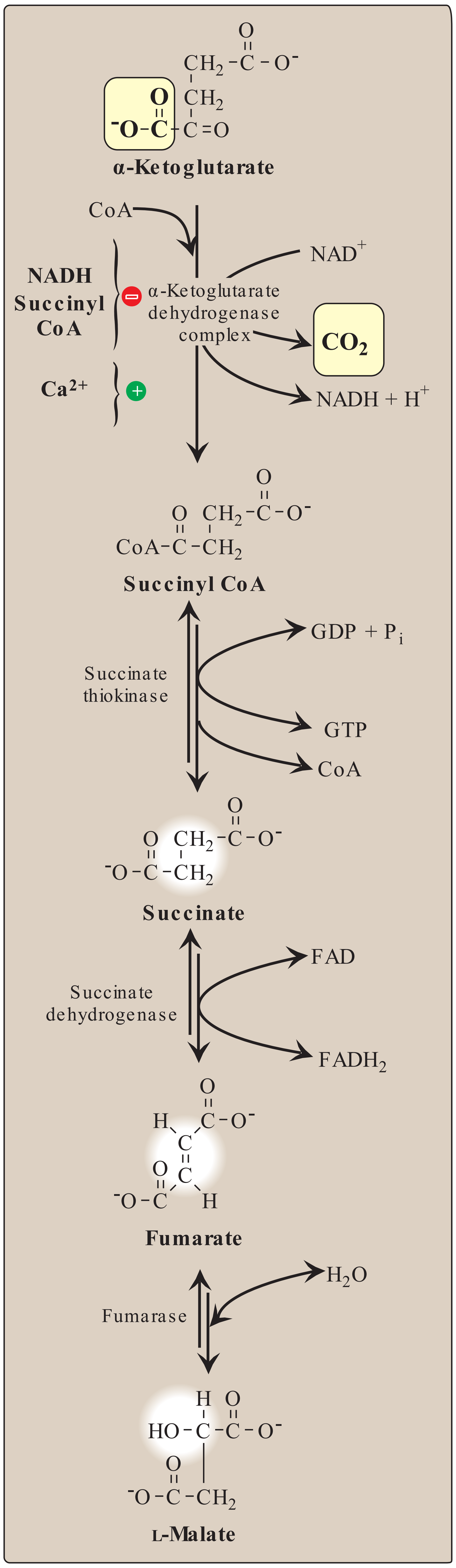


Figure 9.5
Formation of malate from α-ketoglutarate. NAD(H) = nicotinamide adenine dinucleotide; GDP = guanosine diphosphate; P = phosphate; CoA = coenzyme A; FAD(H_2) = flavin adenine dinucleotide.

F. Cleavage of succinyl coenzyme A

Succinate thiokinase (also called succinyl CoA synthetase, named for the reverse reaction) cleaves the high-energy thioester bond of succinyl CoA (see Figure 9.5). This reaction is coupled to phosphorylation of guanosine diphosphate (GDP) to guanosine triphosphate (GTP). GTP and ATP are energetically interconvertible by the nucleoside diphosphate kinase reaction:

$$\text{GTP} + \text{ADP} \rightleftarrows \text{GDP} + \text{ATP}$$

The generation of GTP by succinate thiokinase is another example of substrate-level phosphorylation (see p. 102). [Note: Succinyl CoA is also produced from propionyl CoA derived from the metabolism of fatty acids with an odd number of carbon atoms (see p. 193), and from the metabolism of several amino acids (see pp. 265–266).]

G. Oxidation of succinate

Succinate is oxidized to fumarate by succinate dehydrogenase, as FAD (its coenzyme) is reduced to $FADH_2$ (see Figure 9.5). Succinate dehydrogenase is the only enzyme of the TCA cycle that is embedded in the inner mitochondrial membrane. As such, it functions as Complex II of the electron transport chain (see p. 75). [Note: FAD, rather than NAD^+, is the electron acceptor because the reducing power of succinate is not sufficient to reduce NAD^+.]

H. Hydration of fumarate

Fumarate is hydrated to malate in a freely reversible reaction catalyzed by fumarase (fumarate hydratase; see Figure 9.5). [Note: Fumarate is also produced by the urea cycle (see p. 255), in purine synthesis (see p. 294), and during catabolism of the amino acids phenylalanine and tyrosine (see p. 263).]

I. Oxidation of malate

Malate is oxidized to oxaloacetate by malate dehydrogenase (Figure 9.6). This reaction produces the third and final NADH of the cycle. The standard free energy change (ΔG^0; see p. 70) of the reaction is positive, but the reaction is driven in the direction of OAA by the highly exergonic citrate synthase reaction. [Note: OAA is also produced by the transamination of the amino acid aspartic acid (see p. 250).]

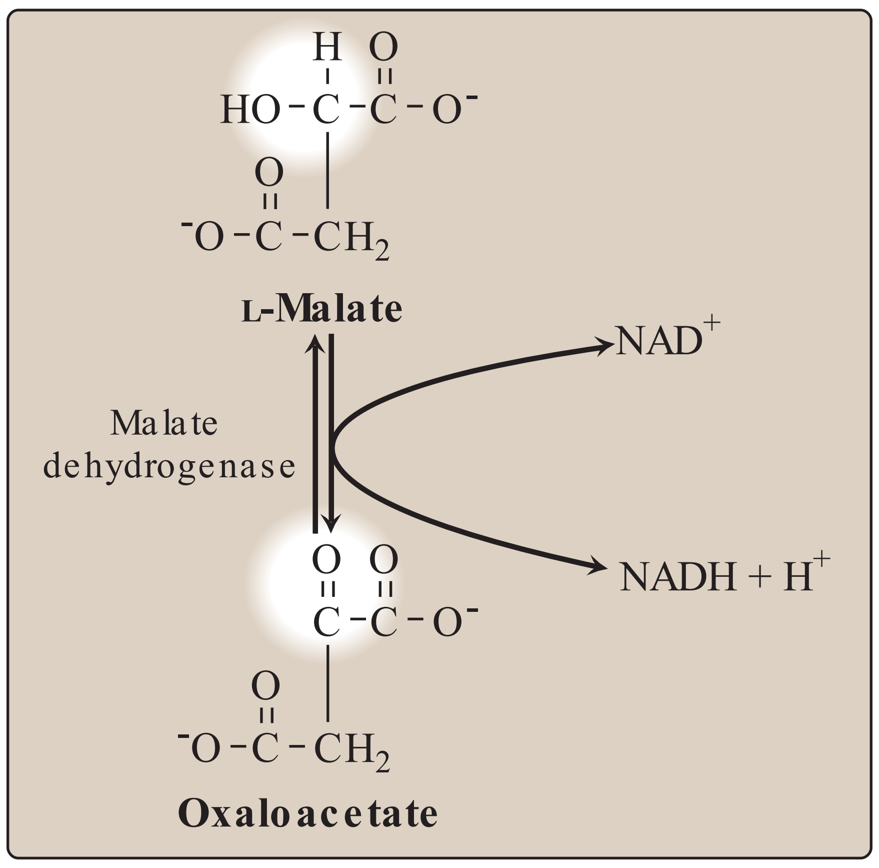


Figure 9.6
Formation (regeneration) of oxaloacetate from malate. NAD(H) = nicotinamide adenine dinucleotide.

III. ENERGY PRODUCED BY THE CYCLE

Two carbon atoms enter the cycle as acetyl CoA and leave as CO_2. The cycle does not involve net consumption or production of OAA or of any other intermediate. Four pairs of electrons are transferred during one turn of the cycle: three pairs of electrons reducing three NAD^+ to NADH and one pair reducing FAD to $FADH_2$. Oxidation of one NADH by the electron transport chain leads to formation of approximately three ATP, whereas oxidation of $FADH_2$ yields approximately two ATP (see p. 77). The total yield of ATP from the oxidation of one acetyl CoA is shown in Figure 9.7. Figure 9.8 summarizes the reactions of the TCA cycle.

Energy-producing reaction	Number of ATP produced
$3\ NADH \longrightarrow 3\ NAD^+$	9
$FADH_2 \longrightarrow FAD$	2
$GDP + P_i \longrightarrow GTP$	1
	12 ATP/acetyl CoA oxidized

Figure 9.7
Number of ATP molecules produced from the oxidation of one molecule of acetyl coenzyme A (CoA) using both substrate-level and oxidative phosphorylation.

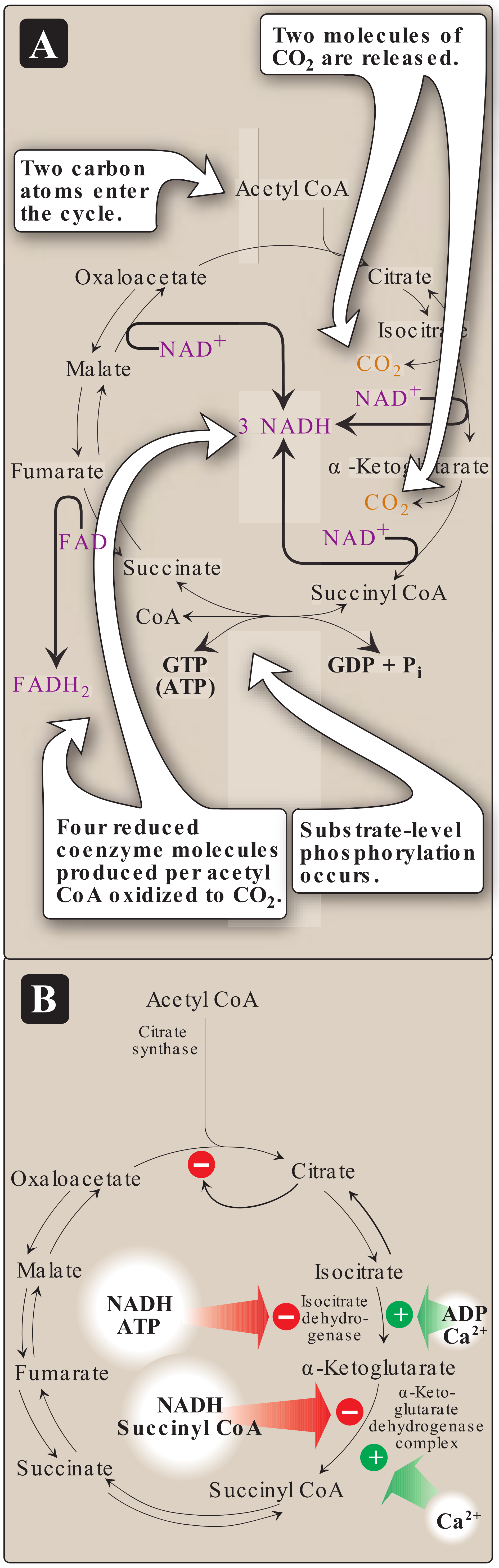

A
Two molecules of CO_2 are released.
Two carbon atoms enter the cycle.
Acetyl CoA
Oxaloacetate
Citrate
Isocitrate
CO_2
NAD$^+$
NAD$^+$
Malate
3 NADH
Fumarate
α -Ketoglutarate
CO_2
NAD$^+$
FAD
Succinate
Succinyl CoA
CoA
GTP (ATP)
GDP + P_i
$FADH_2$
Four reduced coenzyme molecules produced per acetyl CoA oxidized to CO_2.
Substrate-level phosphorylation occurs.
B
Acetyl CoA
Citrate synthase
Oxaloacetate
Citrate
Malate
Isocitrate
NADH ATP
Isocitrate dehydrogenase
ADP Ca^{2+}
Fumarate
α-Ketoglutarate
NADH Succinyl CoA
α-Ketoglutarate dehydrogenase complex
Succinate
Succinyl CoA
Ca^{2+}

Figure 9.8
A. [Note: GTP and ATP are interconverted by nucleoside diphosphate kinase.] Production of reduced coenzymes, ATP, and CO_2 in the citric acid cycle. B. Inhibitors and activators of the cycle.

IV. REGULATION OF THE CYCLE

In contrast to glycolysis, which is regulated primarily by PFK-1, the TCA cycle is controlled by the regulation of several enzymes (see Figure 9.8). The most important of these regulated enzymes are those that catalyze reactions with highly negative ΔG^0: citrate synthase, isocitrate dehydrogenase, and *α*-ketoglutarate dehydrogenase complex. Reducing equivalents needed for oxidative phosphorylation are generated by the PDH complex and the TCA cycle, and both processes are upregulated in response to a decrease in the ratio of ATP to ADP.

V. CHAPTER SUMMARY

Pyruvate is **oxidatively decarboxylated** by pyruvate dehydrogenase (PDH) complex, producing **acetyl coenzyme A (CoA)**, which is the major fuel for the tricarboxylic acid cycle ([TCA cycle] Figure 9.9). This multienzyme complex requires five coenzymes: **thiamine pyrophosphate**, **lipoic acid**, **flavin adenine dinucleotide** (**FAD**), **nicotinamide adenine dinucleotide** (**NAD**$^+$), and **CoA**. PDH complex is regulated by covalent modification of E1 (pyruvate decarboxylase) by PDH kinase and PDH phosphatase: phosphorylation inhibits E1. PDH kinase is allosterically activated by ATP, acetyl CoA, and NADH and inhibited by pyruvate. The phosphatase is activated by Ca^{2+}. PDH complex **deficiency** is the most common biochemical cause of **congenital lactic acidosis**. The central nervous system is particularly affected in this **X-linked dominant** disorder. **Arsenic poisoning** causes inactivation of the PDH complex by binding to lipoic acid. **Citrate** is synthesized from **oxaloacetate** and **acetyl CoA** by citrate synthase. This enzyme is subject to product inhibition by citrate. Citrate is isomerized to **isocitrate** by aconitase (aconitate hydratase). **Isocitrate** is oxidatively decarboxylated by isocitrate dehydrogenase to **α-ketoglutarate**, producing **CO_2** and **NADH**. The enzyme is inhibited by ATP and NADH, and activated by ADP and Ca^{2+}. **α-Ketoglutarate** is oxidatively decarboxylated to **succinyl CoA** by the α-ketoglutarate dehydrogenase complex, producing **CO_2** and **NADH**. The enzyme is very similar to the PDH complex and uses the same coenzymes. *α*-Ketoglutarate dehydrogenase complex is activated by Ca^{+2} and inhibited by NADH and succinyl CoA but is not covalently regulated. **Succinyl CoA** is cleaved by succinate thiokinase (also called succinyl CoA synthetase), producing **succinate** and **GTP**. This is an example of **substrate-level phosphorylation**. **Succinate** is oxidized to **fumarate** by succinate dehydrogenase, producing **$FADH_2$**. **Fumarate** is hydrated to **malate** by fumarase (fumarate hydratase), and **malate** is oxidized to **oxaloacetate** by malate dehydrogenase, producing **NADH**. **Three NADH**, **one $FADH_2$**, and **one GTP** (whose terminal phosphate can be transferred to ADP by nucleoside diphosphate kinase, producing ATP) are produced by one round of the TCA cycle. The generation of acetyl CoA by the oxidation of pyruvate via the PDH complex also produces an NADH. Oxidation of the NADH and $FADH_2$ by the electron transport chain yields 14 ATP. An additional ATP (GTP) comes from substrate level phosphorylation in the TCA cycle. Therefore, a total of 15 ATP are produced from the complete mitochondrial oxidation of pyruvate to CO_2.

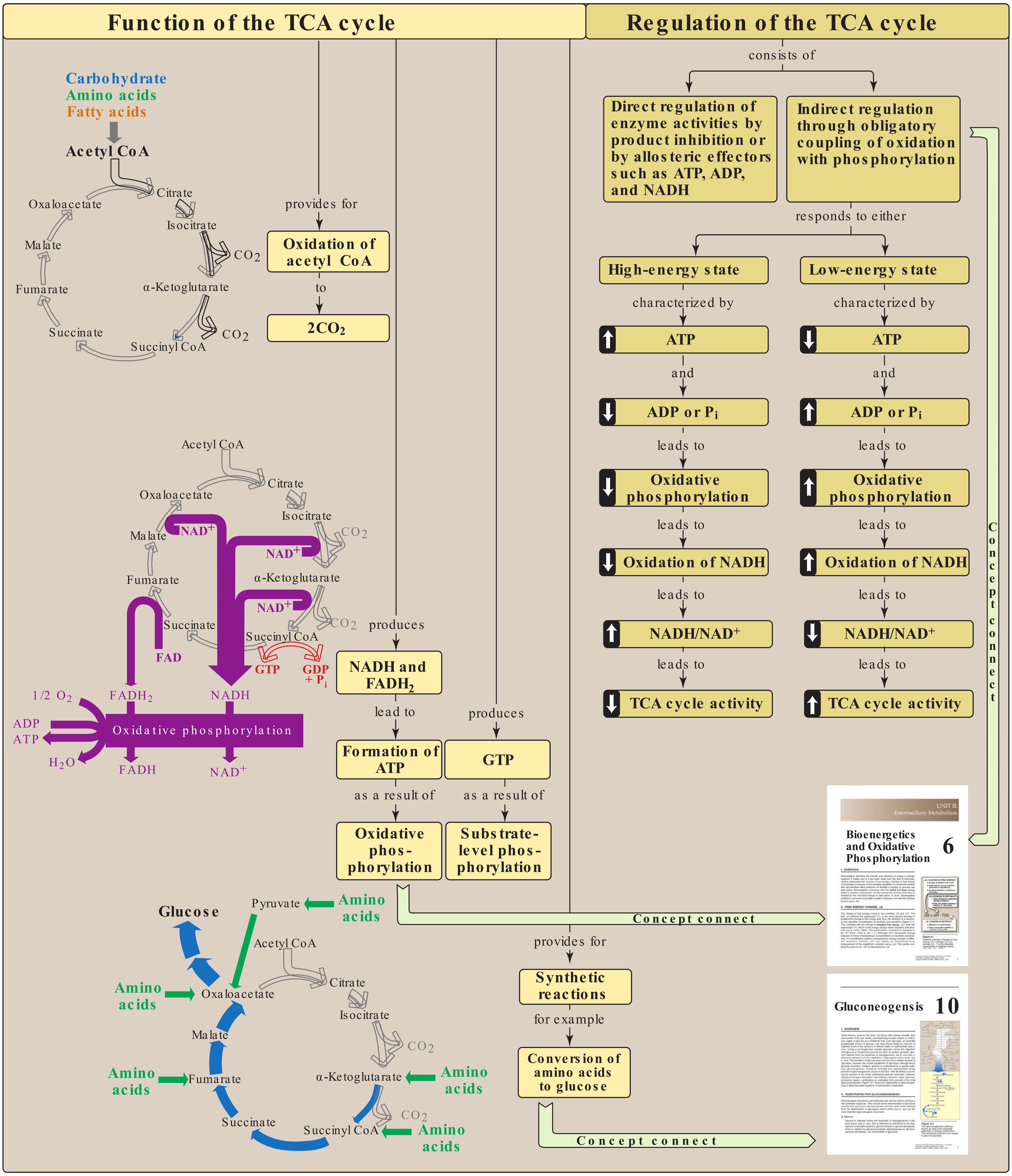

Function of the TCA cycle
Regulation of the TCA cycle
Carbohydrate
Amino acids
Fatty acids
Acetyl CoA
Citrate
Oxaloacetate
Isocitrate
Malate
CO2
Fumarate
α-Ketoglutarate
Succinate
Succinyl CoA
CO2
provides for
Oxidation of acetyl CoA
to
2CO2
produces
NADH and FADH2
lead to
Formation of ATP
as a result of
Oxidative phos-phorylation
produces
GTP
as a result of
Substrate-level phos-phorylation
Acetyl CoA
Citrate
Oxaloacetate
Isocitrate
NAD+
CO2
Malate
NAD+
α-Ketoglutarate
Fumarate
NAD+
CO2
Succinate
Succinyl CoA
FAD
GTP
GDP + Pi
1/2 O2
FADH2
NADH
ADP
ATP
Oxidative phosphorylation
H2O
FADH
NAD+
Concept connect
provides for
Synthetic reactions
for example
Conversion of amino acids to glucose
Concept connect
Glucose
Pyruvate
Amino acids
Acetyl CoA
Amino acids
Oxaloacetate
Citrate
Isocitrate
Malate
CO2
Amino acids
Fumarate
α-Ketoglutarate
Amino acids
Succinate
CO2
Succinyl CoA
Amino acids
consists of
Direct regulation of enzyme activities by product inhibition or by allosteric effectors such as ATP, ADP, and NADH
Indirect regulation through obligatory coupling of oxidation with phosphorylation
responds to either
High-energy state
characterized by
↑ ATP
and
↓ ADP or Pi
leads to
↓ Oxidative phosphorylation
leads to
↓ Oxidation of NADH
leads to
↑ NADH/NAD+
leads to
↓ TCA cycle activity
Low-energy state
characterized by
↓ ATP
and
↑ ADP or Pi
leads to
↑ Oxidative phosphorylation
leads to
↑ Oxidation of NADH
leads to
↓ NADH/NAD+
leads to
↑ TCA cycle activity
Concept connect
UNIT II: Intermediary Metabolism
Bioenergetics and Oxidative Phosphorylation 6
Gluconeogenesis 10

Figure 9.9

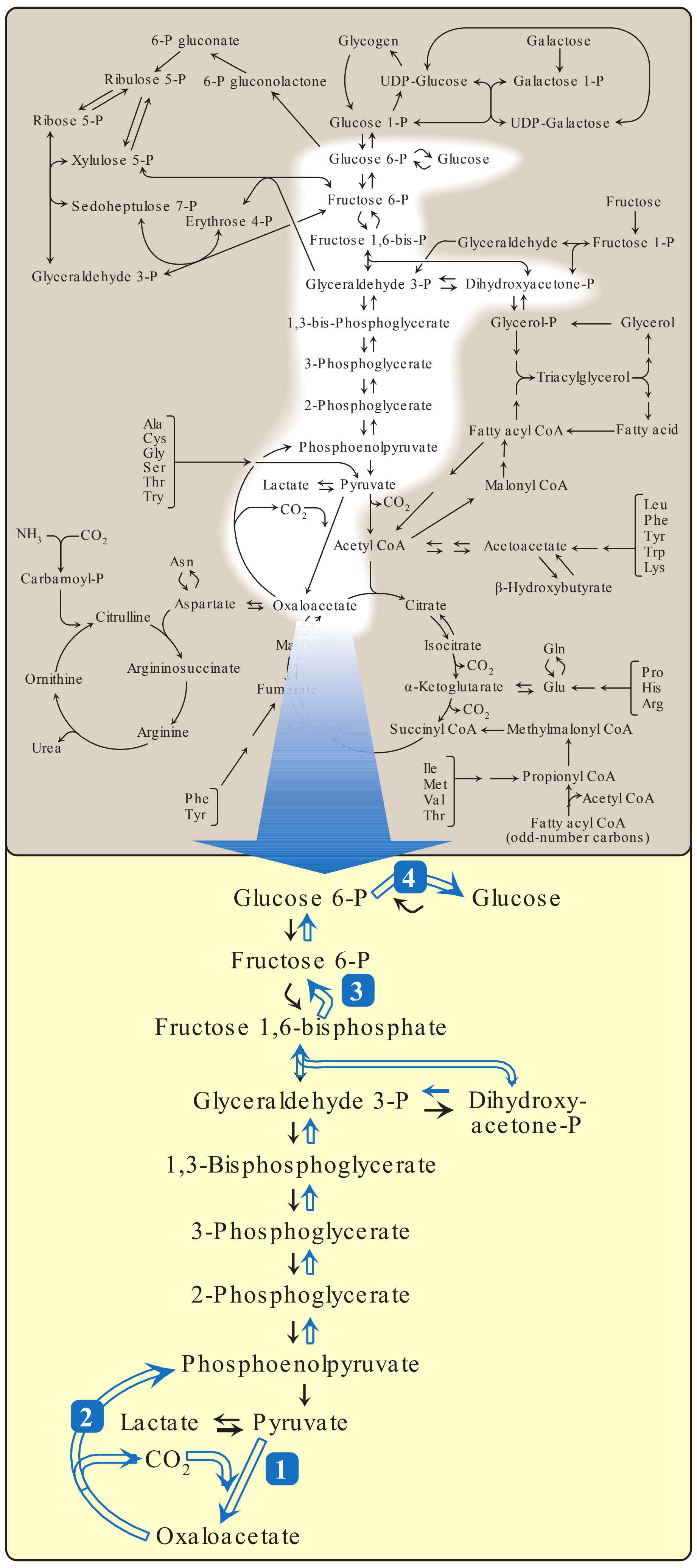
Key concept map for the tricarboxylic acid (TCA) cycle. CoA = coenzyme A; NAD(H) = nicotinamide adenine dinucleotide; FAD(H_2) = flavin adenine dinucleotide; GDP = guanosine diphosphate; GTP = guanosine triphosphate; ADP = adenosine diphosphate; P_i = inorganic phosphate.

Gluconeogenesis

10

I. OVERVIEW

Some tissues, such as the brain, red blood cells (RBCs), kidney medulla, lens and cornea of the eye, testes, and exercising muscle, require a continuous supply of glucose as a metabolic fuel. Liver glycogen, an essential postprandial source of glucose, can meet these needs for only 10–18 hours in the absence of dietary intake of carbohydrate (see p. 329). During a prolonged fast, however, hepatic glycogen stores are depleted, and glucose is formed from noncarbohydrate precursors such as lactate, pyruvate, glycerol (derived from the backbone of triacylglycerols; see p. 190), and α-keto acids (derived from the catabolism of glucogenic amino acids; see p. 261). The formation of glucose does not occur by a simple reversal of glycolysis, because the overall equilibrium of glycolysis strongly favors pyruvate formation. Instead, glucose is synthesized by a special pathway, gluconeogenesis, which requires both mitochondrial and cytosolic enzymes. During an overnight fast, approximately 90% of gluconeogenesis occurs in the liver, with the remaining 10% occurring in the kidneys. However, during prolonged fasting, the kidneys become major glucose-producing organs, contributing an estimated 40% of the total glucose production. Figure 10.1 shows the relationship of gluconeogenesis to other essential pathways of energy metabolism.



6-P gluconate
Glycogen
Galactose
Ribulose 5-P
6-P gluconolactone
UDP-Glucose
Galactose 1-P
Ribose 5-P
Glucose 1-P
UDP-Galactose
Xylulose 5-P
Glucose 6-P
Glucose
Sedoheptulose 7-P
Erythrose 4-P
Fructose 6-P
Fructose
Fructose 1,6-bis-P
Glyceraldehyde
Fructose 1-P
Glyceraldehyde 3-P
Glyceraldehyde 3-P
Dihydroxyacetone-P
1,3-bis-Phosphoglycerate
Glycerol-P
Glycerol
3-Phosphoglycerate
Triacylglycerol
2-Phosphoglycerate
Ala
Cys
Gly
Ser
Thr
Try
Fatty acyl CoA
Fatty acid
Phosphoenolpyruvate
Lactate
Pyruvate
Malonyl CoA
CO_2
CO_2
Leu
Phe
Tyr
Trp
Lys
NH_3
CO_2
Acetyl CoA
Acetoacetate
Carbamoyl-P
Asn
β-Hydroxybutyrate
Aspartate
Oxaloacetate
Citrate
Citrulline
Isocitrate
Gln
CO_2
Pro
His
Arg
Ornithine
Argininosuccinate
α-Ketoglutarate
Glu
CO_2
Urea
Arginine
Succinyl CoA
Methylmalonyl CoA
Ile
Met
Val
Thr
Propionyl CoA
Acetyl CoA
Phe
Tyr
Fatty acyl CoA
(odd-number carbons)
Glucose 6-P
4
Glucose
Fructose 6-P
3
Fructose 1,6-bisphosphate
Glyceraldehyde 3-P
Dihydroxy-
acetone-P
1,3-Bisphosphoglycerate
3-Phosphoglycerate
2-Phosphoglycerate
Phosphoenolpyruvate
2
Lactate
Pyruvate
CO_2
1
Oxaloacetate

Figure 10.1
The gluconeogenesis pathway shown as one of the essential pathways of energy metabolism. The numbered reactions are unique to gluconeogenesis. (See Figure 8.2, p. 92, for a more detailed map of metabolism.) P = phosphate.

II. SUBSTRATES FOR GLUCONEOGENESIS

Gluconeogenic precursors are molecules that can be used to produce a net synthesis of glucose. The most important gluconeogenic prescurors are glycerol, lactate, and the α-keto acids obtained from the metabolism of glucogenic amino acids. [Note: Alanine, which directly gives rise to pyruvate, is an important example of a glucogenic amino acid.]

A. Glycerol

Glycerol is released during the hydrolysis of triacylglycerols in adipose tissue (see p. 190) and is delivered by the blood to the liver. Glycerol is phosphorylated by glycerol kinase to glycerol phosphate, which is oxidized by glycerol phosphate dehydrogenase to dihydroxyacetone phosphate, an intermediate of glycolysis. [Note: Adipocytes cannot phosphorylate glycerol because they essentially lack glycerol kinase.]

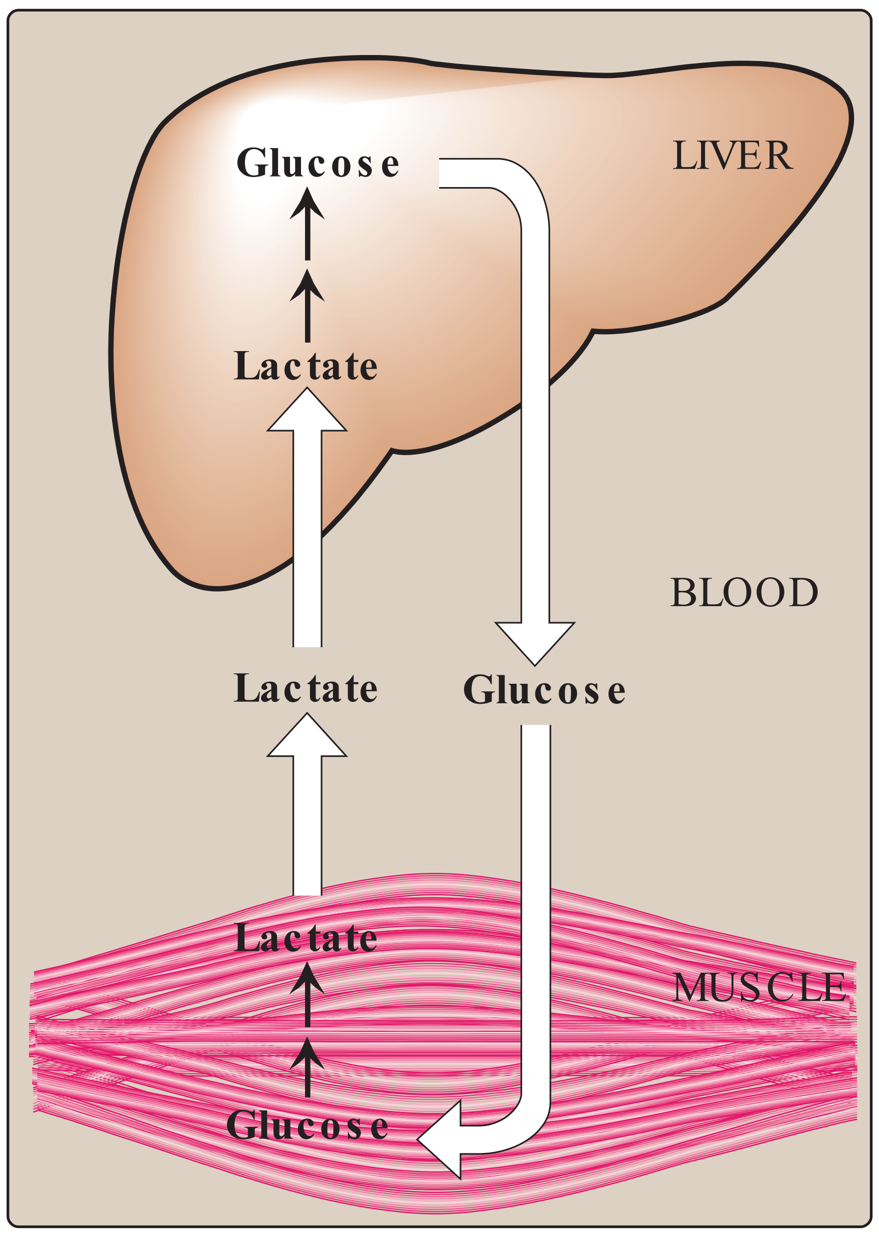


Figure 10.2
The intertissue Cori cycle. [Note: Diffusion of lactate across membranes is facilitated by a transport protein.]

B. Lactate

Lactate is released into the blood by exercising skeletal muscle and by cells that lack mitochondria such as RBCs. In the Cori cycle, bloodborne glucose is converted by exercising muscle to lactate, which diffuses into the blood. This lactate is taken up by the liver and reconverted to glucose, which is released back into the circulation (Figure 10.2).

C. Amino acids

Amino acids derived from hydrolysis of tissue proteins are the major sources of glucose during a fast. The metabolism of the glucogenic amino acids generates α-keto acids. α-Keto acids, such as α-ketoglutarate can enter the tricarboxylic acid (TCA) cycle and form oxaloacetate (OAA), a direct precursor of phosphoenolpyruvate (PEP). [Note: Acetyl coenzyme A (CoA) and compounds that give rise only to acetyl CoA (for example, acetoacetate and amino acids such as lysine and leucine) cannot give rise to a net synthesis of glucose. This is due to the irreversible nature of the pyruvate dehydrogenase (PDH) reaction, which converts pyruvate to acetyl CoA (see p. 109). These compounds give rise instead to ketone bodies (see p. 195) and are, therefore, termed ketogenic.]

III. REACTIONS UNIQUE TO GLUCONEOGENESIS

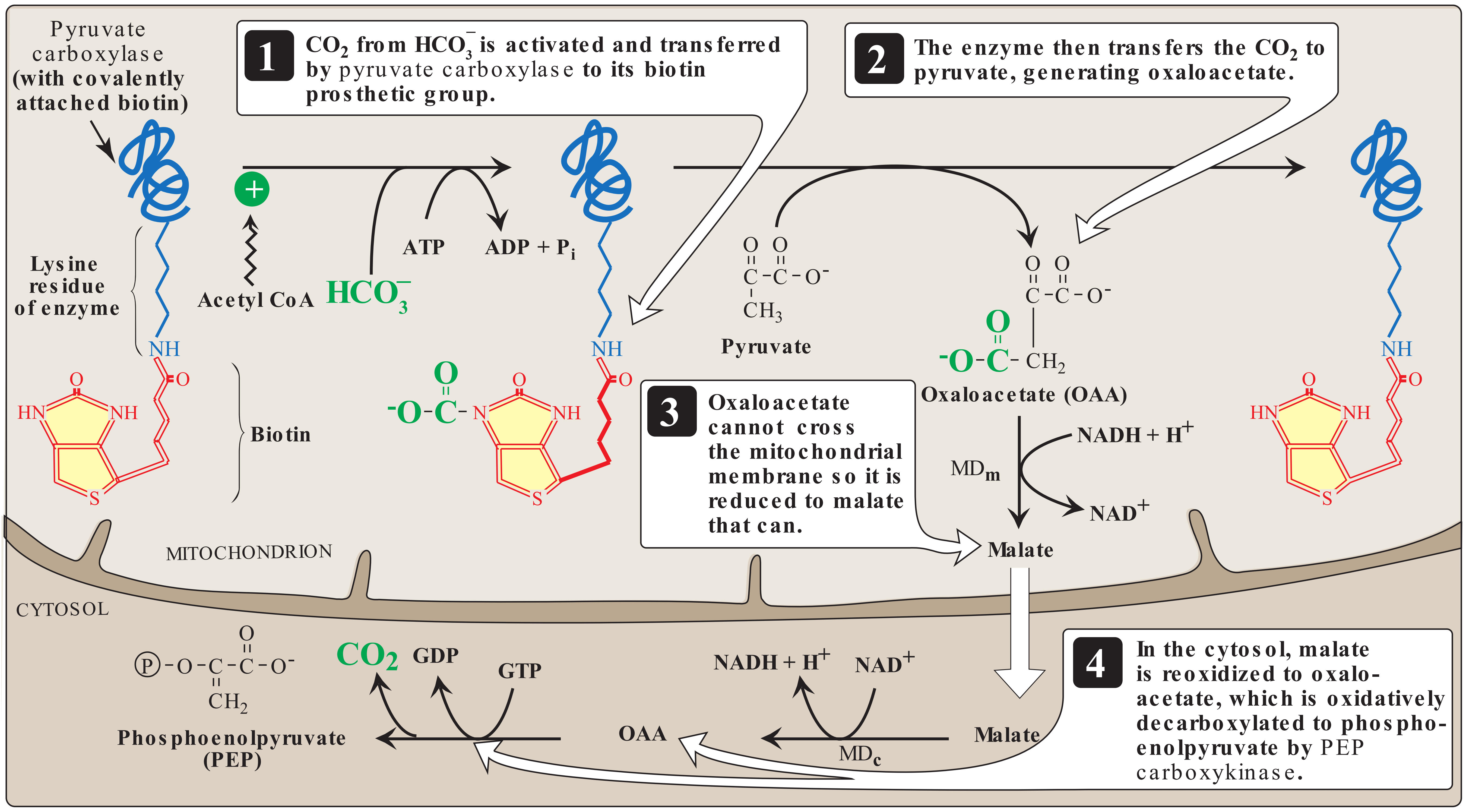
Seven glycolytic reactions are reversible and are used in the synthesis of glucose from lactate or pyruvate. However, three of the reactions are irreversible and must be circumvented by four alternate reactions that energetically favor the synthesis of glucose. These reactions, unique to gluconeogenesis, are described below.

A. Carboxylation of pyruvate

The first "roadblock" to overcome in the synthesis of glucose from pyruvate is the irreversible conversion in glycolysis of PEP to pyruvate by pyruvate kinase (PK). In gluconeogenesis, pyruvate is first carboxylated by pyruvate carboxylase to OAA, which is then converted to PEP by the action of PEP-carboxykinase (Figure 10.3).

1. **Biotin, a coenzyme:** Pyruvate carboxylase requires biotin (see p. 381) covalently bound to the ε-amino group of a lysine residue in the enzyme (see Figure 10.3). Hydrolysis of ATP drives the formation of an enzyme–biotin–CO_2 intermediate, which subsequently carboxylates pyruvate to form OAA. [Note: HCO_3^- is the source of the CO_2.] The pyruvate carboxylase reaction occurs in the mitochondria of liver and kidney cells and has two purposes: to provide an important substrate for gluconeogenesis and to provide OAA that can replenish the TCA cycle intermediates that may become depleted, depending on the synthetic needs of the cell. Muscle cells also contain pyruvate carboxylase but use the OAA produced only for the replenishment (anaplerotic) purpose and do not synthesize glucose.

> Pyruvate carboxylase is one of several carboxylases that require biotin. Others include acetyl CoA carboxylase (p. 183), propionyl CoA carboxylase (p. 193), and methylcrotonyl CoA carboxylase (p. 266).


Pyruvate carboxylase (with covalently attached biotin)
Lysine residue of enzyme
NH
O
HN
NH
S
Biotin
Acetyl CoA
+
HCO_3^-
ATP
ADP + P_i
$^-O-C-$
Pyruvate
Oxaloacetate (OAA)
MD_m
NADH + H^+
NAD^+
Malate
MITOCHONDRION
CYTOSOL
Phosphoenolpyruvate (PEP)
CO_2
GDP
GTP
OAA
NADH + H^+
NAD^+
MD_c
Malate

1 CO_2 from HCO_3^- is activated and transferred by pyruvate carboxylase to its biotin prosthetic group.

2 The enzyme then transfers the CO_2 to pyruvate, generating oxaloacetate.

3 Oxaloacetate cannot cross the mitochondrial membrane so it is reduced to malate that can.

4 In the cytosol, malate is reoxidized to oxaloacetate, which is oxidatively decarboxylated to phosphoenolpyruvate by PEP carboxykinase.

Figure 10.3
Carboxylation of pyruvate to OAA, followed by reduction of OAA to malate for transfer to the cystol and subsequent decarboxylation to PEP. [Note: OAA can also be converted to PEP or aspartate for transfer to the cytosol.] MD_m = mitochondrial malate dehydrogenase; MD_c = cytosolic malate dehydrogenase.

2. **Allosteric regulation:** Pyruvate carboxylase is allosterically activated by acetyl CoA. Elevated levels of acetyl CoA in mitochondria signal a metabolic state in which the increased synthesis of OAA is required. For example, this occurs during fasting, when OAA is used for the synthesis of glucose by gluconeogenesis in the liver and kidney. Conversely, at low levels of acetyl CoA, pyruvate carboxylase is largely inactive, and pyruvate is primarily oxidized by the PDH complex to produce acetyl CoA that can be further oxidized by the TCA cycle (see p. 109).

B. Transport of oxaloacetate to the cytosol

OAA must be converted to PEP for gluconeogenesis to continue. The enzyme that catalyzes this reaction is found in both the mitochondria and the cytosol in humans. The PEP generated in the mitochondria is transported to the cytosol by a specific transporter, whereas that generated in the cytosol requires the transport of OAA from the mitochondria to the cytosol. However, OAA is unable to be transported across the inner mitochondrial membrane, so it must first be reduced to malate by mitochondrial malate dehydrogenase (MD). Malate can be transported from the mitochondria to the cytosol, where it is reoxidized to OAA by cytosolic MD as nicotinamide adenine dinucleotide (NAD^+) is reduced (see Figure 10.3). The NADH produced is used in the reduction of 1,3-bisphosphoglycerate to glyceraldehyde 3-phosphate (see p. 101), a step common to both glycolysis and gluconeogenesis. [Note: OAA also can be converted to aspartate, which is transported out of the mitochondria.]

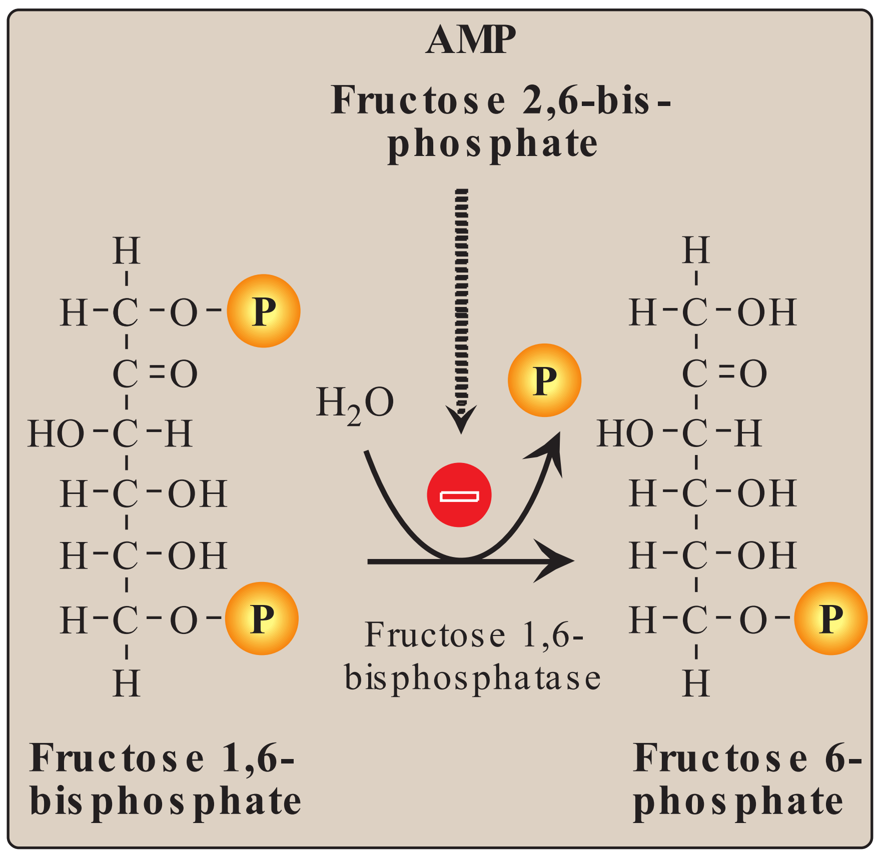

AMP
Fructose 2,6-bis-phosphate
H
H-C-O-P
C=O
HO-C-H
H-C-OH
H-C-OH
H-C-O-P
H
H_2O
P
Fructose 1,6-bisphosphatase
H
H-C-OH
C=O
HO-C-H
H-C-OH
H-C-OH
H-C-O-P
H
Fructose 1,6-bisphosphate
Fructose 6-phosphate

Figure 10.4
Dephosphorylation of fructose 1,6-bisphosphate. AMP = adenosine monophosphate; P = phosphate.

C. Decarboxylation of cytosolic oxaloacetate

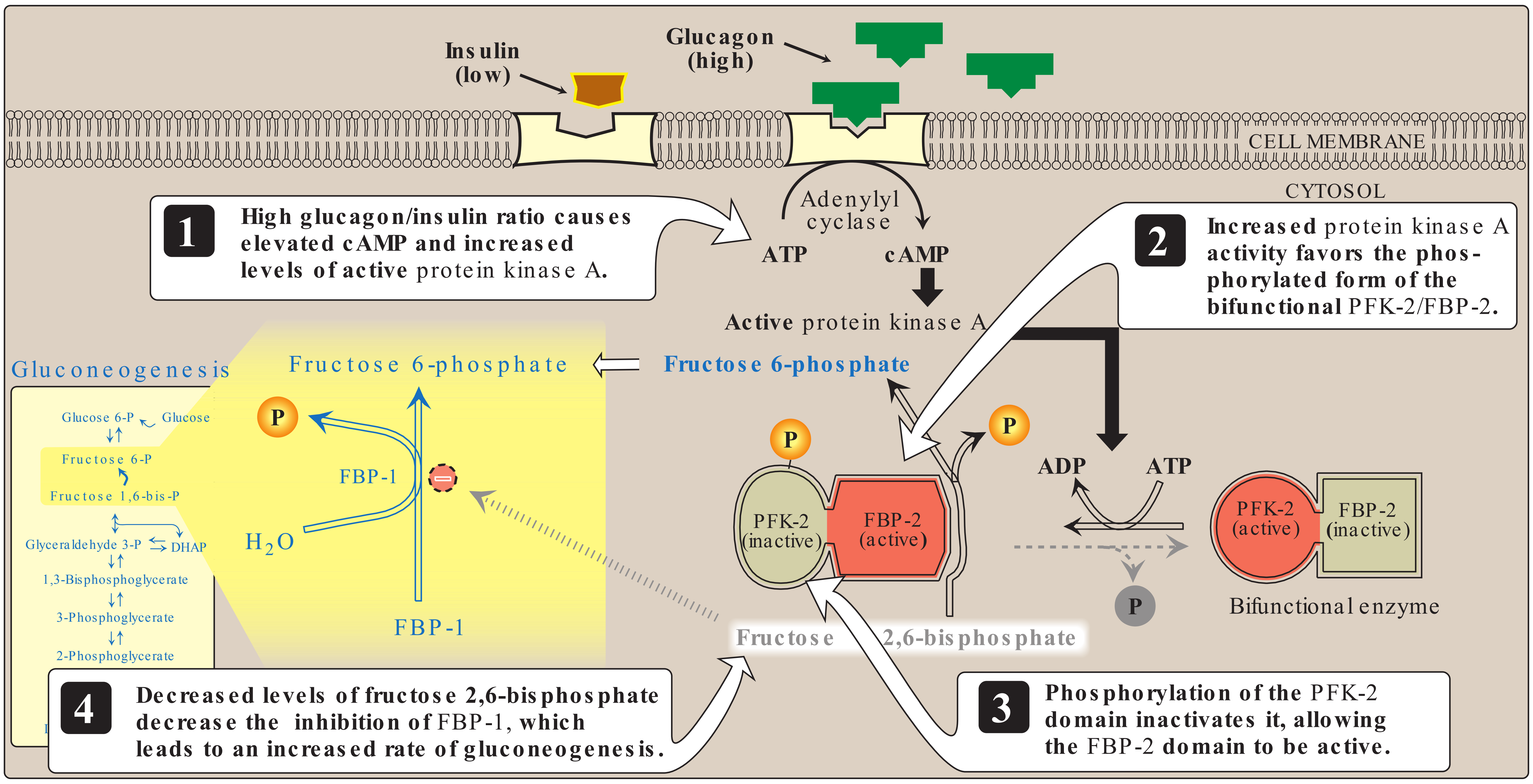
OAA is decarboxylated and phosphorylated to PEP in the cytosol by PEP-carboxykinase (also referred to as PEPCK). The reaction is driven by hydrolysis of guanosine triphosphate ([GTP] see Figure 10.3). The combined actions of pyruvate carboxylase and PEP-carboxykinase provide an energetically favorable pathway from pyruvate to PEP. PEP is then acted on by the reactions of glycolysis running in the reverse direction until it becomes fructose 1,6-bisphosphate.

> The pairing of carboxylation with decarboxylation, as seen in gluconeogenesis, drives reactions that would otherwise be energetically unfavorable. A similar strategy is used in fatty acid synthesis (see pp. 183–184).

D. Dephosphorylation of fructose 1,6-bisphosphate

Hydrolysis of fructose 1,6-bisphosphate by fructose 1,6-bisphosphatase, found in liver and kidney, bypasses the irreversible phosphofructokinase-1 (PFK-1) reaction, and provides an energetically favorable pathway for the formation of fructose 6-phosphate (Figure 10.4). This reaction is an important regulatory site of gluconeogenesis.

1. **Regulation by energy levels within the cell:** Fructose 1,6-bisphosphatase is inhibited by elevated levels of adenosine monophos


Insulin (low)
Glucagon (high)
CELL MEMBRANE
CYTOSOL
Adenylyl cyclase
ATP
cAMP
Active protein kinase A
Gluconeogenesis
Glucose 6-P
Glucose
Fructose 6-P
Fructose 1,6-bis-P
Glyceraldehyde 3-P
DHAP
1,3-Bisphosphoglycerate
3-Phosphoglycerate
2-Phosphoglycerate
Fructose 6-phosphate
P
FBP-1
H_2O
FBP-1
Fructose 6-phosphate
P
P
PFK-2 (inactive)
FBP-2 (active)
ADP
ATP
P
PFK-2 (active)
FBP-2 (inactive)
Bifunctional enzyme
Fructose 2,6-bisphosphate

1 **High glucagon/insulin ratio causes elevated cAMP and increased levels of active protein kinase A.**

2 **Increased protein kinase A activity favors the phosphorylated form of the bifunctional PFK-2/FBP-2.**

3 **Phosphorylation of the PFK-2 domain inactivates it, allowing the FBP-2 domain to be active.**

4 **Decreased levels of fructose 2,6-bisphosphate decrease the inhibition of FBP-1, which leads to an increased rate of gluconeogenesis.**

Figure 10.5
Effect of elevated glucagon on the intracellular concentration of fructose 2,6-bisphosphate in the liver. cAMP = cyclic AMP; PFK-2 = phosphofructokinase-2; FBP-2 = fructose 2,6-bisphosphatase; FBP-1 = fructose 1,6-bisphosphatase; P = phosphate.

phate (AMP), which signal an "energy-poor" state in the cell. Conversely, high levels of ATP and low concentrations of AMP stimulate gluconeogenesis, an energy-requiring pathway.

2. **Regulation by fructose 2,6-bisphosphate:** Fructose 1,6-bisphosphatase is inhibited by fructose 2,6-bisphosphate, an allosteric effector whose concentration is influenced by the insulin to glucagon ratio: when glucagon is high, the effector is not made and, thus, the phosphatase is active. (Figure 10.5). [Note: The signals that inhibit (low energy, high fructose 2,6-bisphosphate) or activate (high energy, low fructose 2,6-bisphosphate) gluconeogenesis have the opposite effect on glycolysis, providing reciprocal control of the pathways that synthesize and oxidize glucose (see p. 100).]

E. Dephosphorylation of glucose 6-phosphate

Hydrolysis of glucose 6-phosphate by glucose 6-phosphatase bypasses the irreversible hexokinase/glucokinase reaction and provides an energetically favorable pathway for the formation of free glucose (Figure 10.6). Liver and kidney are the only organs that release free glucose from glucose 6-phosphate. This process actually requires a complex of two proteins: glucose 6-phosphate translocase, which transports glucose 6-phosphate across the endoplasmic reticular (ER) membrane, and the enzyme glucose 6-phosphatase (found only in gluconeogenic cells), which removes the phosphate, producing free glucose (see Figure 10.6). [Note: These ER-membrane proteins are also required for the final step of glycogen degradation (see p. 130). Type Ia and Ib glycogen storage disease, caused by deficiencies in the phosphatase and the transferase, respectively, are characterized by severe fasting hypoglycemia, because free glucose is unable to be produced from either gluconeogenesis or glycogenolysis.] Specific glucose transporters (GLUTs) are responsible for moving free glucose into the cytosol and then into blood. [Note: Glucose 6-phosphate translocase moves inorganic phosphate out of the ER as it moves glucose 6-phosphate in.]

F. Summary of the reactions of glycolysis and gluconeogenesis

Of the 11 reactions required to convert pyruvate to free glucose, 7 are catalyzed by reversible glycolytic enzymes (Figure 10.7). The irreversible reactions of glycolysis catalyzed by hexokinase/glucokinase, PFK-1, and PK are circumvented by glucose 6-phosphatase, fructose 1,6-bisphosphatase, and pyruvate carboxylase/PEP-carboxykinase. In gluconeogenesis, the equilibria of the 7 reversible reactions of glycolysis are pushed in favor of glucose synthesis as a result of the essentially irreversible formation of PEP, fructose 6-phosphate, and glucose catalyzed by the gluconeogenic enzymes. [Note: The stoichiometry of gluconeogenesis from pyruvate couples the cleavage of six high-energy phosphate bonds and the oxidation of two NADH with the formation of each molecule of glucose (see Figure 10.7).]

IV. REGULATION OF GLUCONEOGENESIS

The moment-to-moment regulation of gluconeogenesis is determined primarily by the circulating level of glucagon and by the availability of gluconeogenic substrates. In addition, slow adaptive changes in

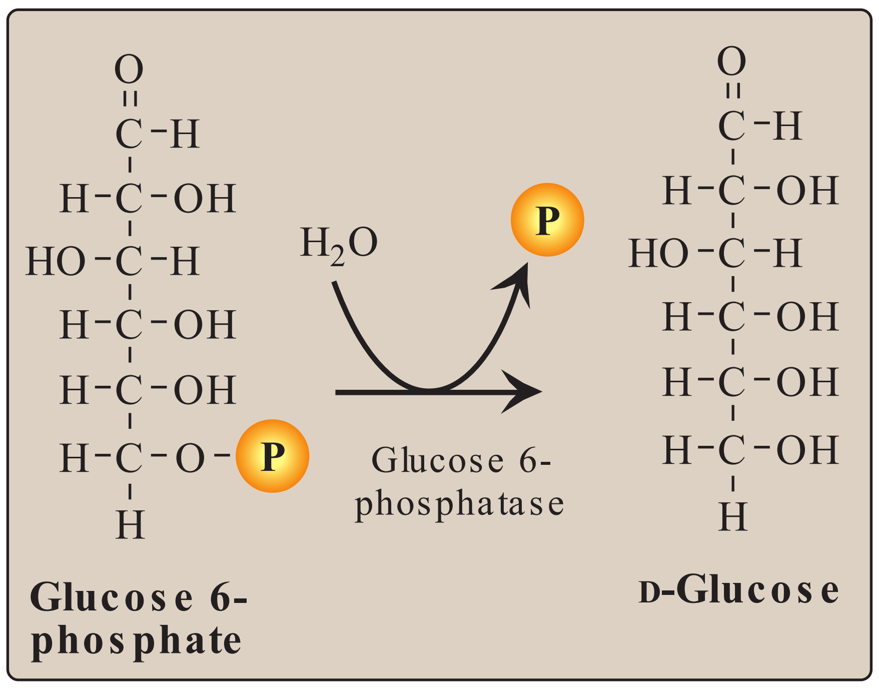


Figure 10.6
Dephosphorylation of glucose 6-phosphate allows release of free glucose from the liver and kidney into blood. P = phosphate.

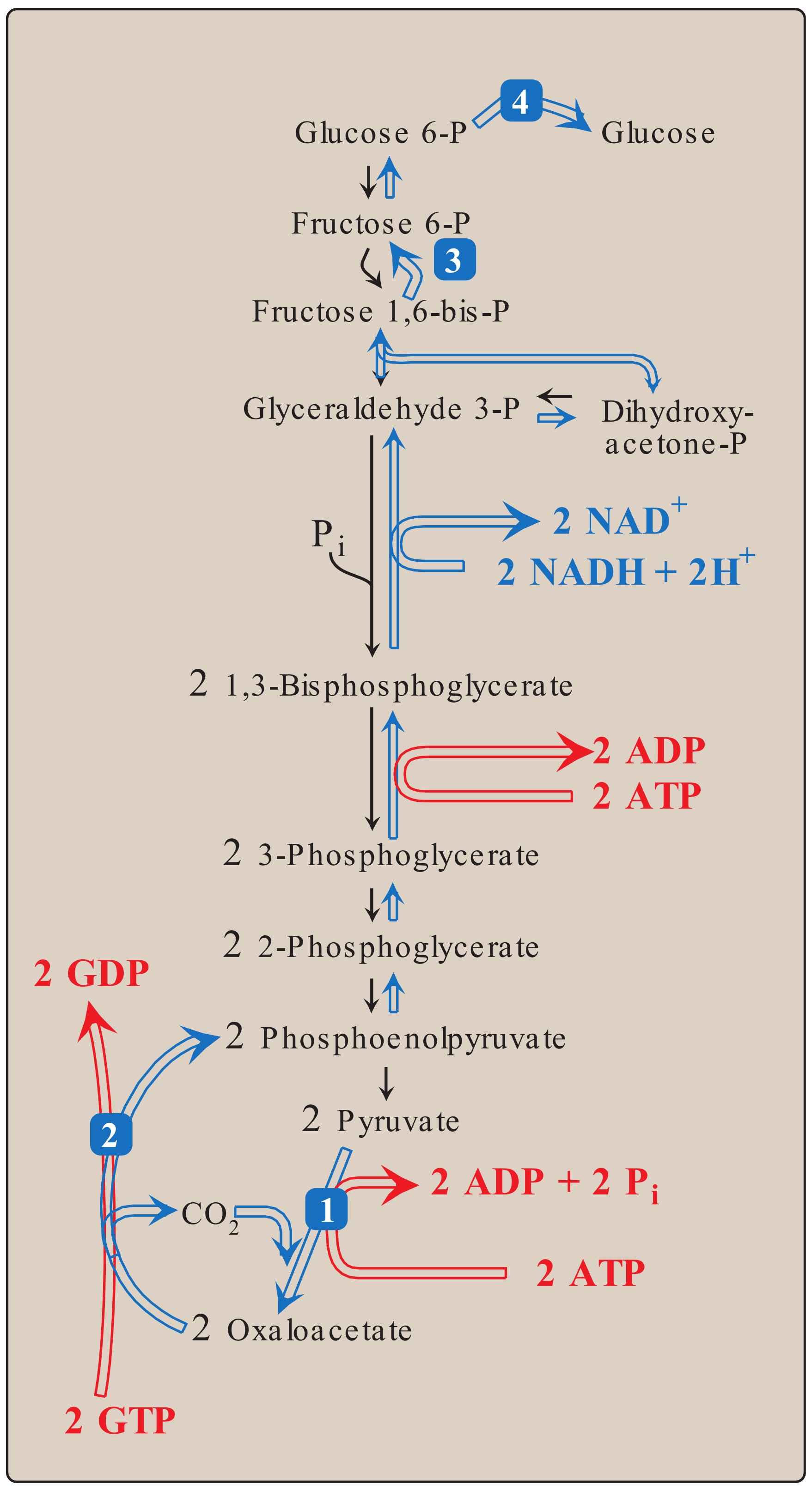


Figure 10.7
Summary of the reactions of glycolysis and gluconeogenesis, showing the energy requirements of gluconeogenesis. The numbered reactions are unique to gluconeogenesis. P = phosphate; GDP = guanosine diphosphate; GTP = guanosine triphosphate; NAD(H) = nicotinamide adenine dinucleotide.

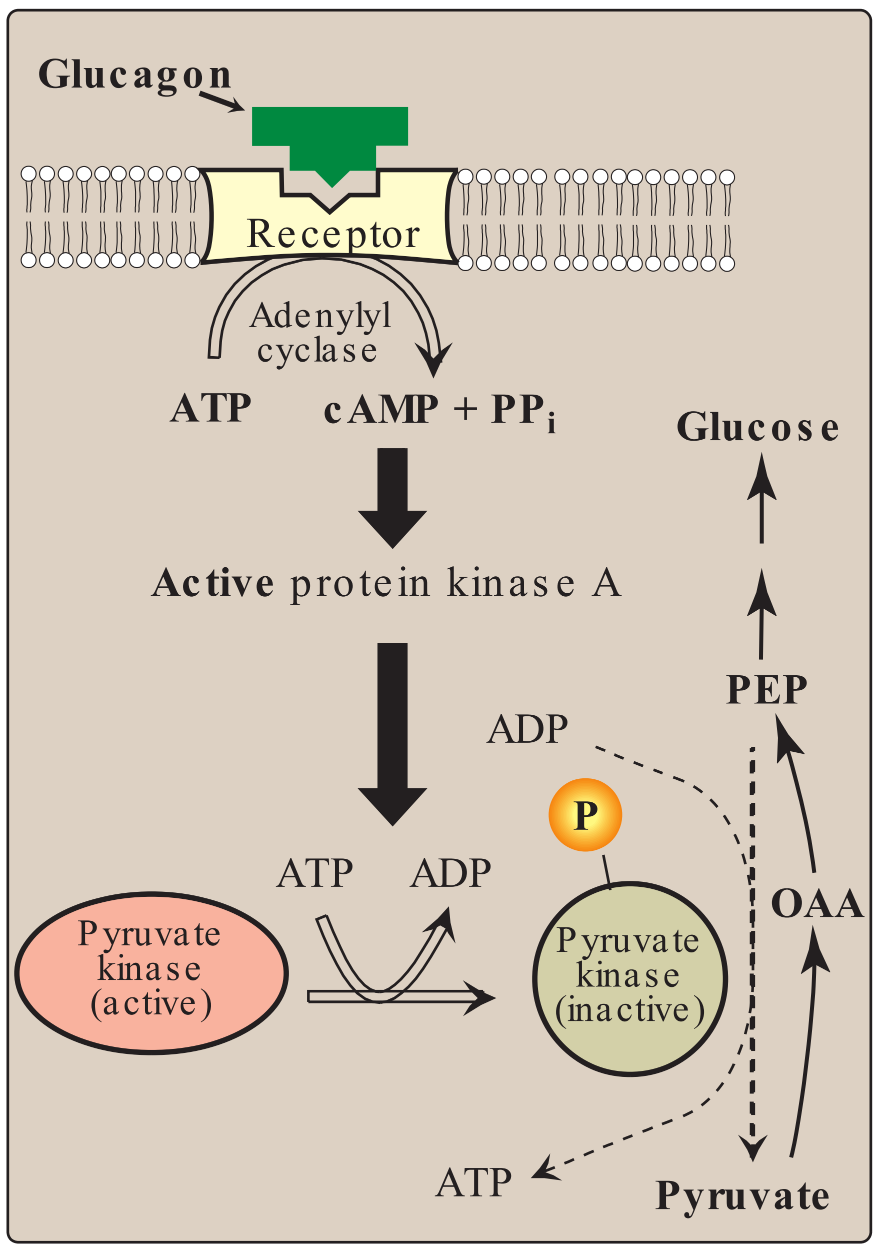


Figure 10.8
Covalent modification of pyruvate kinase results in inactivation of the enzyme. [Note: Only the hepatic isozyme is subject to covalent regulation.] OAA = oxaloacetate; PEP = phosphoenolpyruvate; cAMP = cyclic AMP; PP$_i$ = pyrophosphate; P = phosphate.

enzyme activity result from an alteration in the rate of enzyme synthesis or degradation or both. [Note: Hormonal control of the glucoregulatory system is presented in Chapter 23.]

A. Glucagon

This peptide hormone from the α cells of pancreatic islets (see p. 313) stimulates gluconeogenesis by three mechanisms.

1. **Changes in allosteric effectors:** Glucagon lowers the level of fructose 2,6-bisphosphate, resulting in activation of fructose 1,6-bisphosphatase and inhibition of PFK-1, thus favoring gluconeogenesis over glycolysis (see Figure 10.5). [Note: See p. 99 for the role of fructose 2,6-bisphosphate in the regulation of glycolysis.]
2. **Covalent modification of enzyme activity:** Glucagon binds its G protein–coupled receptor (see p. 95) and, via an elevation in cyclic AMP (cAMP) level and cAMP-dependent protein kinase activity, stimulates the conversion of hepatic PK to its inactive (phosphorylated) form. This decreases the conversion of PEP to pyruvate, which has the effect of diverting PEP to the synthesis of glucose (Figure 10.8).
3. **Induction of enzyme synthesis:** Glucagon increases the transcription of the gene for PEP-carboxykinase, thereby increasing the availability of this enzyme as levels of its substrate rise during fasting. [Note: Glucocorticoids also increase expression of the gene, whereas insulin decreases expression.]

B. Substrate availability

The availability of gluconeogenic precursors, particularly glucogenic amino acids, significantly influences the rate of glucose synthesis. Decreased levels of insulin favor mobilization of amino acids from muscle protein and provide the carbon skeletons for gluconeogenesis. The ATP and NADH coenzymes-cosubstrates required for gluconeogenesis are primarily provided by the catabolism of fatty acids.

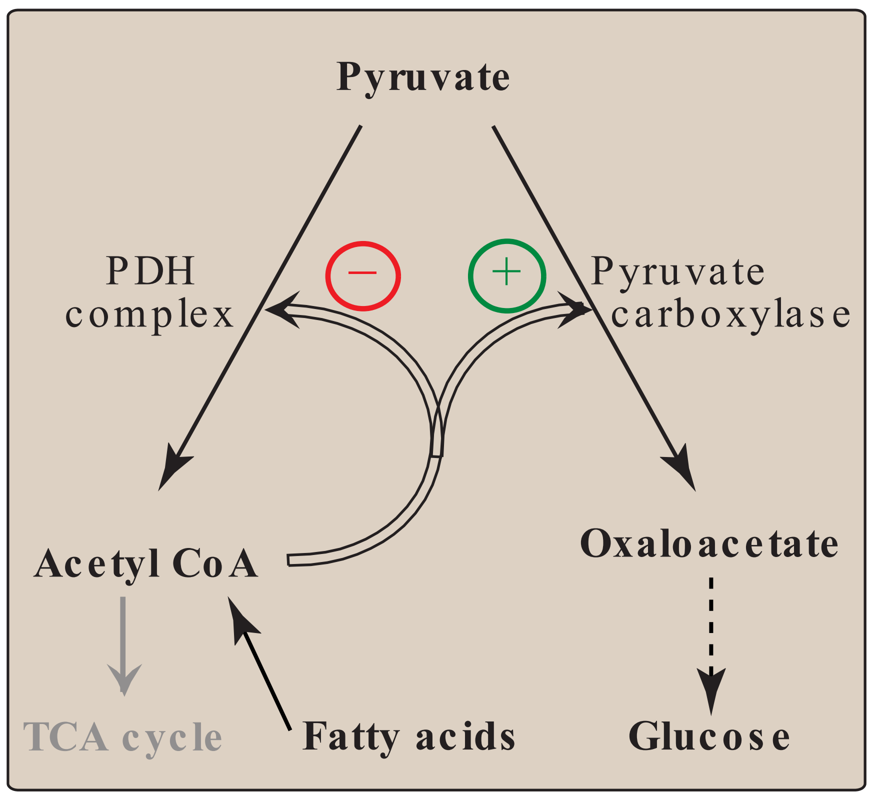


Figure 10.9
Acetyl coenzyme A (CoA) diverts pyruvate away from oxidation and toward gluconeogenesis. PDH = pyruvate dehydrogenase; TCA = tricarboxylic acid.

C. Allosteric activation by acetyl coenzyme A

Allosteric activation of hepatic pyruvate carboxylase by acetyl CoA occurs during fasting. As a result of increased lipolysis in adipose tissue, the liver is flooded with fatty acids (see p. 330). The rate of formation of acetyl CoA by β-oxidation of these fatty acids exceeds the capacity of the liver to oxidize it to CO_2 and H_2O. As a result, acetyl CoA accumulates and activates pyruvate carboxylase. [Note: Acetyl CoA inhibits the PDH complex (by activating PDH kinase; see p. 111). Thus, this single compound can divert pyruvate toward gluconeogenesis and away from the TCA cycle (Figure 10.9).]

D. Allosteric inhibition by adenosine monophosphate

Fructose 1,6-bisphosphatase is inhibited by AMP—a compound that activates PFK-1. This results in a reciprocal regulation of glycolysis and gluconeogenesis seen previously with fructose 2,6-bisphosphate (see p. 121). [Note: Elevated AMP, thus, stimulates pathways that oxidize nutrients to provide energy for the cell.]

V. CHAPTER SUMMARY

Gluconeogenic precursors include the **intermediates of glycolysis** and the **tricarboxylic acid cycle**, **glycerol** released during the hydrolysis of triacylglycerols in adipose tissue, **lactate** released by cells that lack mitochondria and by exercising skeletal muscle, and **α-keto acids** derived from the metabolism of glucogenic amino acids (Figure 10.10). Seven of the reactions of glycolysis are reversible and are used for gluconeogenesis in the liver and kidneys. Three reactions are **physiologically irreversible** and must be circumvented. These reactions are catalyzed by the glycolytic enzymes pyruvate kinase, phosphofructokinase, and hexokinase. **Pyruvate** is converted to oxaloacetate and then to **phosphoenolpyruvate** (**PEP**) by pyruvate carboxylase and PEP-carboxykinase. The carboxylase requires **biotin** and **ATP** and is allosterically activated by **acetyl coenzyme A**. PEP-carboxykinase requires **GTP**. The transcription of its gene is increased by glucagon and the glucocorticoids and decreased by insulin. **Fructose 1,6-bisphosphate** is converted to **fructose 6-phosphate** by fructose 1,6-bisphosphatase. This enzyme is **inhibited** by elevated levels of **AMP** and **activated** when **ATP** levels are elevated. The enzyme is also **inhibited** by **fructose 2,6-bisphosphate**, the primary allosteric activator of glycolysis. **Glucose 6-phosphate** is converted to **glucose** by glucose 6-phosphatase. This enzyme of the endoplasmic reticular membrane is required for the final step in gluconeogenesis as well as hepatic and renal glycogen degradation. Its deficiency results in severe, fasting hypoglycemia.

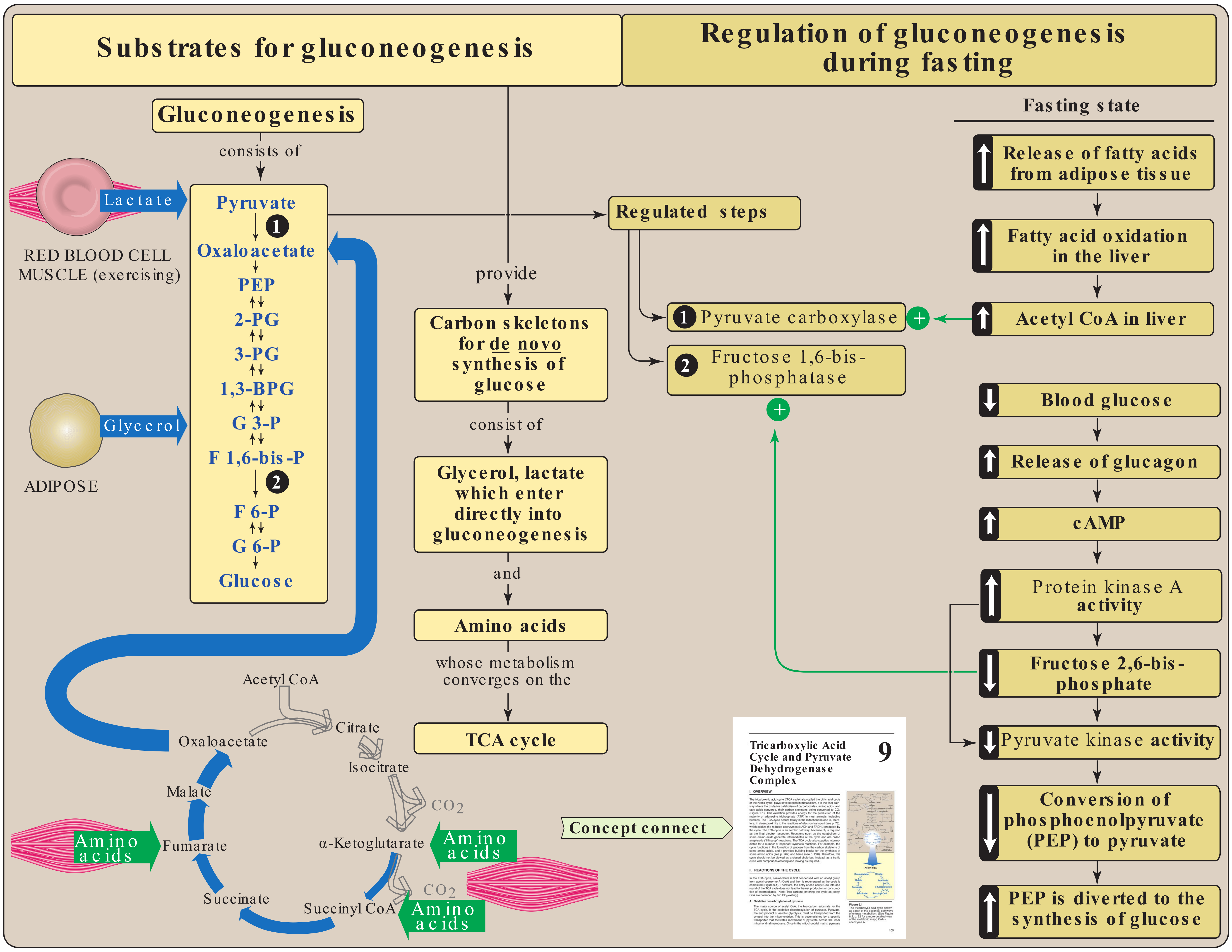

Substrates for gluconeogenesis
Regulation of gluconeogenesis during fasting
Gluconeogenesis
consists of
Lactate
RED BLOOD CELL
MUSCLE (exercising)
Glycerol
ADIPOSE
Pyruvate
1
Oxaloacetate
PEP
2-PG
3-PG
1,3-BPG
G 3-P
F 1,6-bis-P
2
F 6-P
G 6-P
Glucose
provide
Carbon skeletons for de novo synthesis of glucose
consist of
Glycerol, lactate which enter directly into gluconeogenesis
and
Amino acids
whose metabolism converges on the
TCA cycle
Acetyl CoA
Citrate
Oxaloacetate
Isocitrate
Malate
CO2
Amino acids
Fumarate
α-Ketoglutarate
Amino acids
Succinate
Succinyl CoA
CO2
Amino acids
Regulated steps
1 Pyruvate carboxylase
+
2 Fructose 1,6-bisphosphatase
+
Fasting state
Release of fatty acids from adipose tissue
Fatty acid oxidation in the liver
Acetyl CoA in liver
Blood glucose
Release of glucagon
cAMP
Protein kinase A activity
Fructose 2,6-bisphosphate
Pyruvate kinase activity
Conversion of phosphoenolpyruvate (PEP) to pyruvate
PEP is diverted to the synthesis of glucose
Concept connect
Tricarboxylic Acid Cycle and Pyruvate Dehydrogenase Complex
9

Figure 10.10
Key concept map for gluconeogenesis. TCA = tricarboxylic acid. CoA = coenzyme A; cAMP = cyclic adenosine monophosphate; P = phosphate; PG = phosphoglycerate; BPG = bisphosphoglycerate.

Glycogen Metabolism

11

I. OVERVIEW

A constant source of blood glucose is an absolute requirement for human life. Glucose is the greatly preferred energy source for the brain, and the required energy source for cells with few or no mitochondria such as mature red blood cells. Glucose is also essential as an energy source for exercising muscle, where it is the substrate for anaerobic glycolysis. Blood glucose can be obtained from three primary sources: the diet, degradation of glycogen, and gluconeogenesis. Dietary intake of glucose and glucose precursors, such as starch (a polysaccharide), disaccharides, and monosaccharides, is sporadic and, depending on the diet, is not always a reliable source of blood glucose. In contrast, gluconeogenesis (see p. 117) can provide sustained synthesis of glucose, but it is somewhat slow in responding to a falling blood glucose level. Therefore, the body has developed mechanisms for storing a supply of glucose in a rapidly mobilizable form, namely, glycogen. In the absence of a dietary source of glucose, this sugar is rapidly released from liver and kidney glycogen. Similarly, muscle glycogen is extensively degraded in exercising muscle to provide that tissue with an important energy source. When glycogen stores are depleted, specific tissues synthesize glucose de novo, using amino acids from the body's proteins as a primary source of carbons for the gluconeogenic pathway. Figure 11.1 shows the reactions of glycogen synthesis and degradation as part of the essential pathways of energy metabolism.

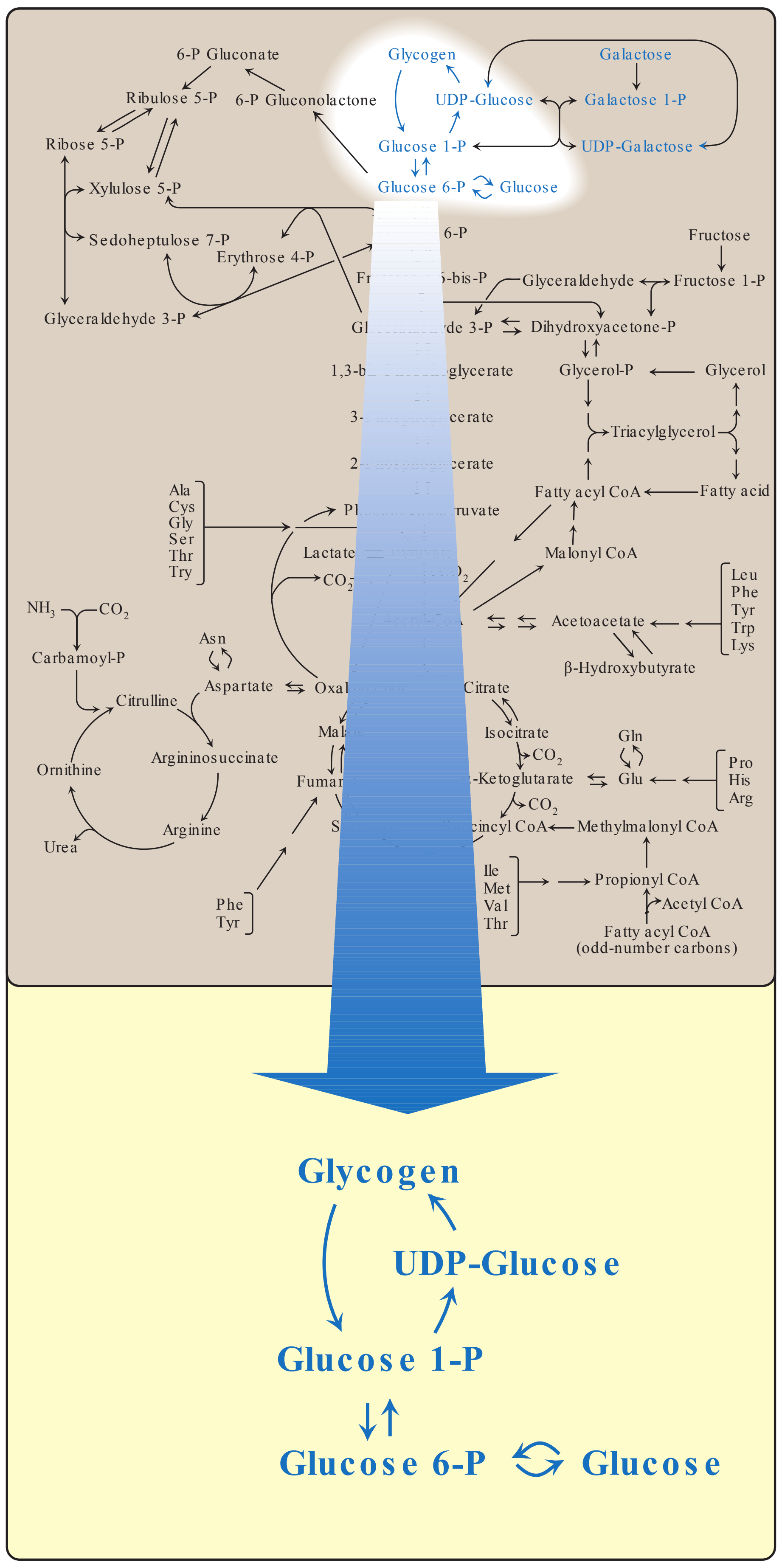

6-P Gluconate
Glycogen
Galactose
Ribulose 5-P
6-P Gluconolactone
UDP-Glucose
Galactose 1-P
Ribose 5-P
Glucose 1-P
UDP-Galactose
Xylulose 5-P
Glucose 6-P
Glucose
Sedoheptulose 7-P
Erythrose 4-P
Fructose
Glyceraldehyde
Fructose 1-P
Glyceraldehyde 3-P
Dihydroxyacetone-P
Glycerol-P
Glycerol
Triacylglycerol
Fatty acyl CoA
Fatty acid
Malonyl CoA
Ala
Cys
Gly
Ser
Thr
Try
Lactate
Leu
Phe
Tyr
Trp
Lys
NH_3
CO_2
Carbamoyl-P
Asn
Acetoacetate
β-Hydroxybutyrate
Aspartate
Citrate
Citrulline
Isocitrate
Gln
Argininosuccinate
Glu
Pro
His
Arg
Ornithine
Methylmalonyl CoA
Urea
Arginine
Ile
Met
Val
Thr
Propionyl CoA
Acetyl CoA
Phe
Tyr
Fatty acyl CoA
(odd-number carbons)
Glycogen
UDP-Glucose
Glucose 1-P
Glucose 6-P
Glucose

Figure 11.1
Glycogen synthesis and degradation shown as a part of the essential pathways of energy metabolism (see Figure 8.2, p. 92, for a more detailed view of the overall reactions of metabolism). P = phosphate; UDP = uridine diphosphate.

II. STRUCTURE AND FUNCTION OF GLYCOGEN

The main stores of glycogen are found in skeletal muscle and liver, although most other cells store small amounts of glycogen for their own use. The function of muscle glycogen is to serve as a fuel reserve for the synthesis of adenosine triphosphate (ATP) during muscle contraction. That of liver glycogen is to maintain the blood glucose concentration, particularly during the early stages of a fast (Figure 11.2; also see p. 329). [Note: Liver glycogen can maintain blood glucose for 10–18 hours.]

A. Amounts of liver and muscle glycogen

Approximately 400 g of glycogen make up 1%–2% of the fresh weight of resting muscle, and approximately 100 g of glycogen make up to 10% of the fresh weight of a well-fed adult liver. What limits the production of glycogen at these levels is not clear. However, in some

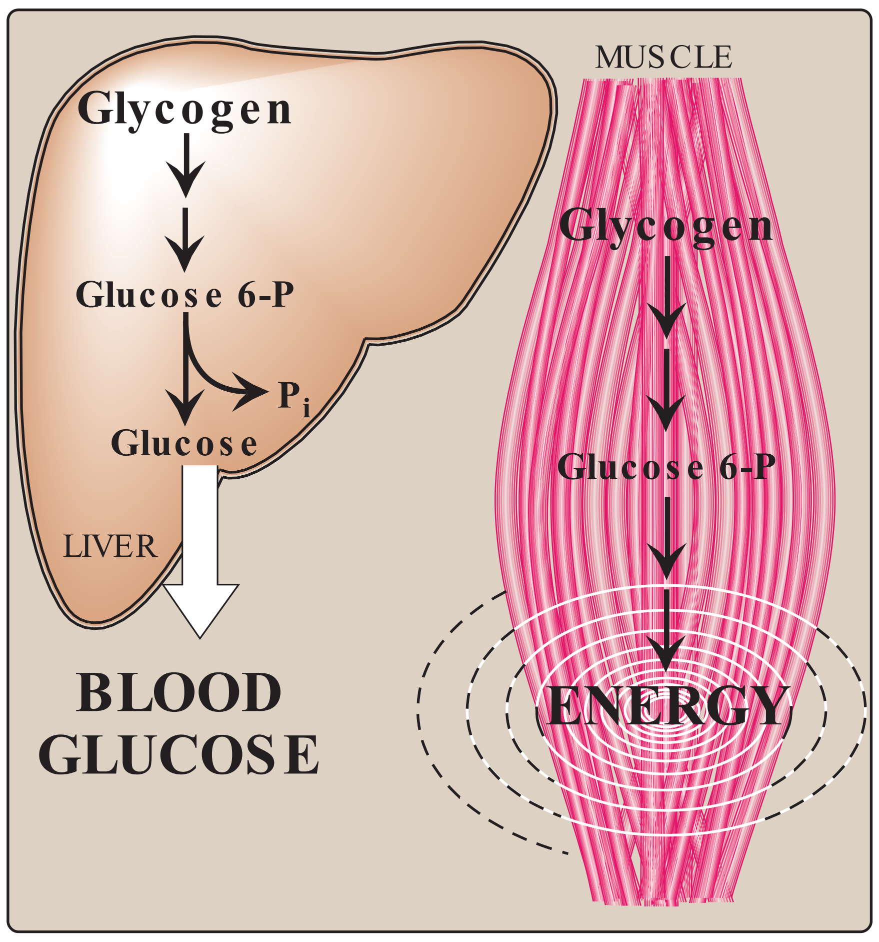


Figure 11.2
Functions of muscle and liver glycogen. P = phosphate; P_i = inorganic phosphate.

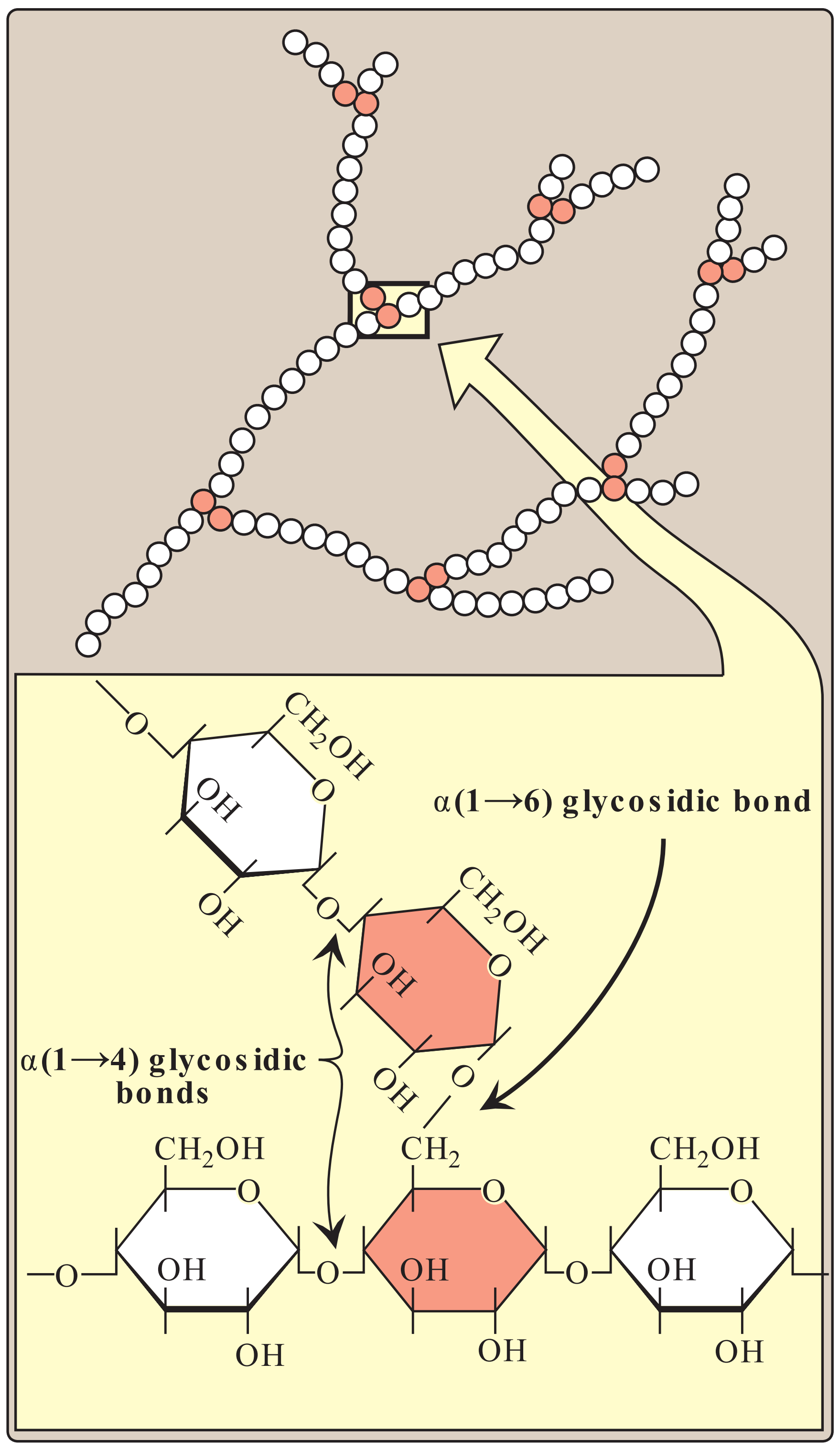


Figure 11.3
Branched structure of glycogen, showing α(1→4) and α(1→6) glycosidic bonds.

glycogen storage diseases ([GSDs] see Figure 11.8), the amount of glycogen in the liver and/or muscle can be significantly higher. [Note: In the body, muscle mass is greater than liver mass. Consequently, most of the body's glycogen is found in muscle.]

B. Structure of glycogen

Glycogen is a branched-chain polysaccharide made exclusively from α-D-glucose. The primary glycosidic bond is an α(1→4) linkage. After an average of eight to ten glucosyl residues, there is a branch containing an α(1→6) linkage (Figure 11.3). A single glycogen molecule can have a molecular weight of up to 10^8 Da. These polymers of glucose exist in discrete cytoplasmic granules that also contain most of the enzymes necessary for glycogen synthesis and degradation.

C. Fluctuation of glycogen stores

Liver glycogen stores increase during the well-fed state (see p. 323) and are depleted during a fast (see p. 329). Muscle glycogen is not affected by short periods of fasting (a few days) and is only moderately decreased in prolonged fasting (weeks). Muscle glycogen is synthesized to replenish muscle stores after they have been depleted following strenuous exercise. [Note: Glycogen synthesis and degradation go on continuously. The differences between the rates of these two processes determine the levels of stored glycogen during specific physiologic states.]

III. SYNTHESIS OF GLYCOGEN (GLYCOGENESIS)

Glycogen is synthesized from molecules of α-D-glucose. The process occurs in the cytosol and requires energy supplied by ATP (for the phosphorylation of glucose) and uridine triphosphate (UTP).

A. Synthesis of uridine diphosphate glucose

α-D-Glucose attached to uridine diphosphate (UDP) is the source of all the glucosyl residues that are added to the growing glycogen molecule. UDP-glucose (Figure 11.4) is synthesized from glucose 1-phosphate and UTP by UDP-glucose pyrophosphorylase (Figure 11.5). Pyrophosphate (PP_i), the second product of the reaction, is hydrolyzed to two inorganic phosphates (P_i) by pyrophosphatase. The hydrolysis is exergonic, ensuring that the UDP-glucose pyrophosphorylase reaction proceeds in the direction of UDP-glucose production. [Note: Glucose 1-phosphate is generated from glucose 6-phosphate by phosphoglucomutase. Glucose 1,6-bisphosphate is an obligatory intermediate in this reversible reaction (Figure 11.6).]

B. Synthesis of a primer to initiate glycogen synthesis

Glycogen synthase makes the α(1→4) linkages in glycogen. This enzyme cannot initiate chain synthesis using free glucose as an acceptor of a molecule of glucose from UDP-glucose. Instead, it can only elongate already existing chains of glucose and, therefore, requires a primer. A fragment of glycogen can serve as a primer in cells whose glycogen stores are not totally depleted. In the absence of a glycogen fragment, a protein called glycogenin can serve as an acceptor of glucose residues from UDP-glucose (see Figure 11.5). The side-chain hydroxyl group of a specific tyrosine in the protein serves as the site at which the initial glucosyl unit is attached. Because the reaction is catalyzed by glycogenin itself via autoglucosylation, glycogenin is an enzyme. Glycogenin then catalyzes the transfer of the next few molecules of glucose from UDP-glucose, producing a short, α(1→4)-linked glucosyl chain. This short chain serves as a primer that is able to be elongated by glycogen synthase as described below [Note: Glycogenin stays associated with and forms the core of a glycogen granule.]

C. Elongation of glycogen chains by glycogen synthase

Elongation of a glycogen chain involves the transfer of glucose from UDP-glucose to the nonreducing end of the growing chain, forming a new glycosidic bond between the anomeric hydroxyl group of carbon 1 of the activated glucose and carbon 4 of the accepting glucosyl residue (see Figure 11.5). [Note: The nonreducing end of a carbohydrate chain is one in which the anomeric carbon of the terminal sugar is linked by a glycosidic bond to another compound, making the terminal sugar nonreducing (see p. 84).] The enzyme responsible for making the α(1→4) linkages in glycogen is glycogen synthase. [Note: The UDP released when the new α(1→4) glycosidic bond is made can be phosphorylated to UTP by nucleoside diphosphate kinase (UDP + ATP ⇄ UTP + ADP; see p. 296).]

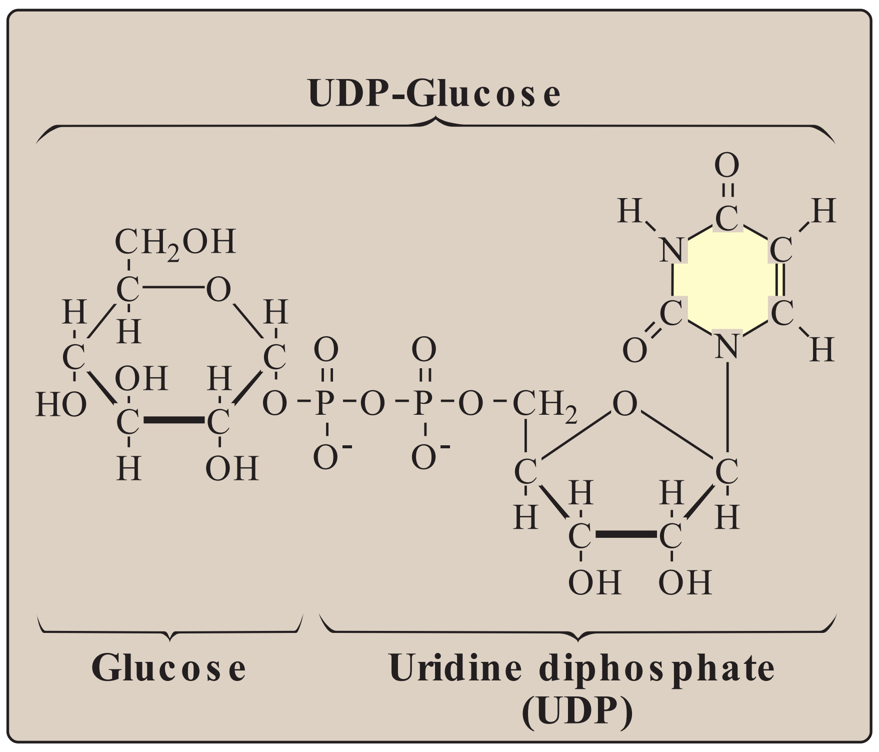

UDP-Glucose
Glucose
Uridine diphosphate (UDP)

Figure 11.4
The structure of UDP-glucose, a nucleotide sugar.

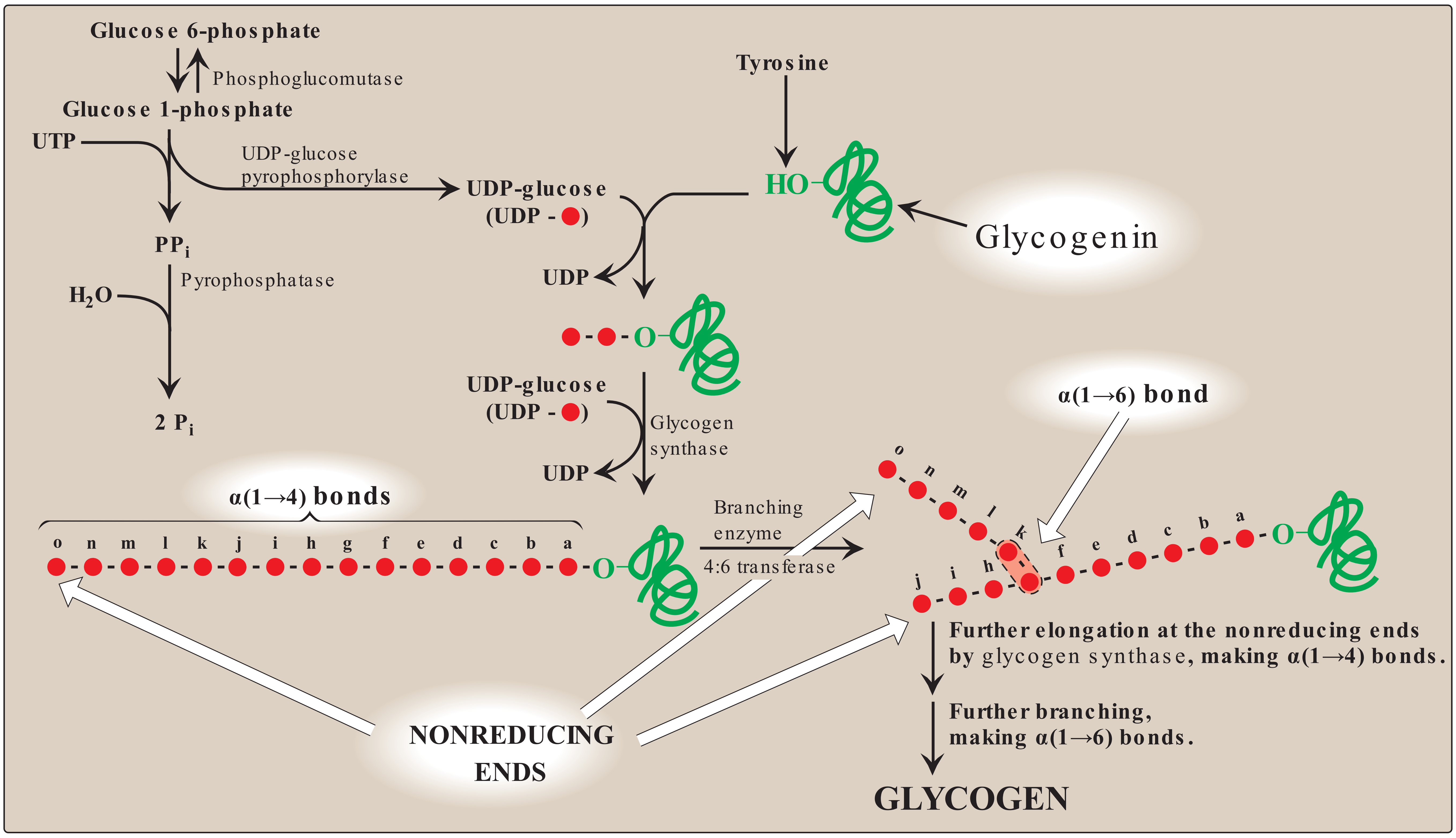

Glucose 6-phosphate
Phosphoglucomutase
Glucose 1-phosphate
UTP
UDP-glucose pyrophosphorylase
UDP-glucose (UDP - ●)
PP$_i$
Pyrophosphatase
H$_2$O
2 P$_i$
Tyrosine
HO-
Glycogenin
UDP
O
UDP-glucose (UDP - ●)
Glycogen synthase
UDP
α(1→4) bonds
o n m l k j i h g f e d c b a
Branching enzyme
4:6 transferase
α(1→6) bond
o n m l k
j i h
f e d c b a
Further elongation at the nonreducing ends by glycogen synthase, making α(1→4) bonds.
Further branching, making α(1→6) bonds.
GLYCOGEN
NONREDUCING ENDS

Figure 11.5
Glycogen synthesis. UTP = uridine triphosphate; UDP = uridine diphosphate; PP$_i$ = pyrophosphate; P$_i$ = inorganic phosphate.

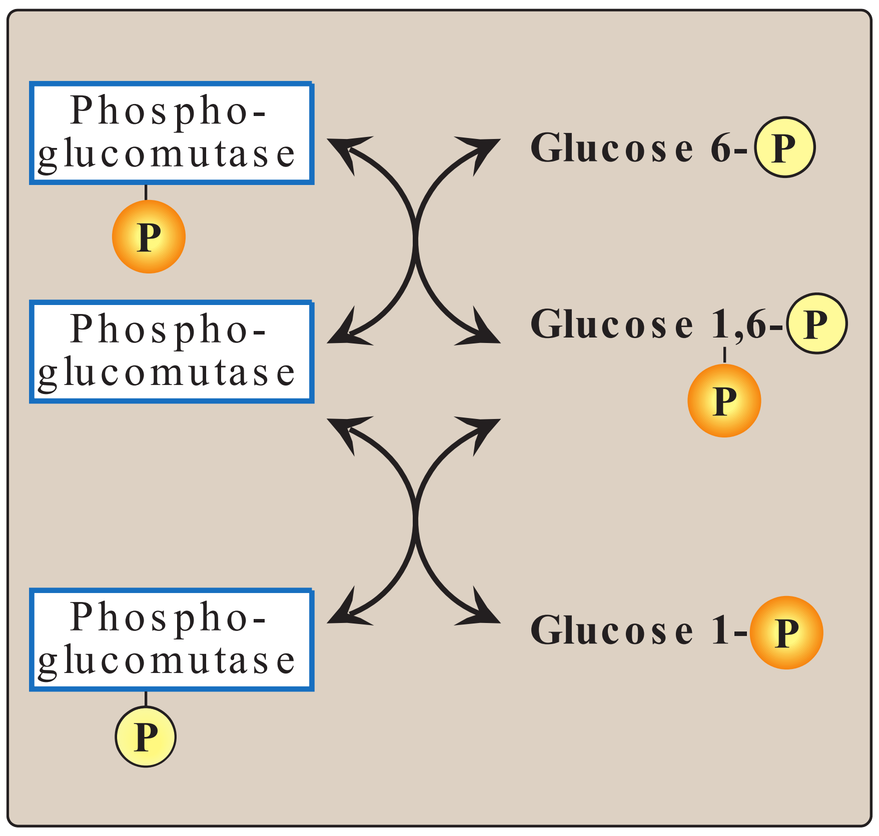


Figure 11.6
Interconversion of glucose 6-phosphate and glucose 1-phosphate by phosphoglucomutase. P = phosphate.

D. Formation of branches in glycogen

If no other synthetic enzyme acted on the chain, the resulting structure would be a linear (unbranched) chain of glucosyl residues attached by α(1→4) linkages. Such a compound is found in plant tissues and is called amylose. In contrast, glycogen has branches located, on average, eight glucosyl residues apart, resulting in a highly branched, tree-like structure (see Figure 11.3) that is far more soluble than the unbranched amylose. Branching also increases the number of nonreducing ends to which new glucosyl residues can be added (and also, as described later, from which these residues can be removed), thereby greatly accelerating the rate at which glycogen synthesis can occur and dramatically increasing the size of the glycogen molecule.

1. **Synthesis of branches:** Branches are made by the action of the branching enzyme, amylo-*α*(1→4)→*α*(1→6)-transglucosidase. This enzyme removes a set of six to eight glucosyl residues from the nonreducing end of the glycogen chain, breaking an α(1→4) bond to another residue on the chain, and attaches it to a nonterminal glucosyl residue by an α(1→6) linkage, thus functioning as a 4:6 transferase. The resulting new, nonreducing end (see "j" in Figure 11.5), as well as the old nonreducing end from which the six to eight residues were removed (see "o" in Figure 11.5), can now be further elongated by glycogen synthase.

2. **Synthesis of additional branches:** After elongation of these two ends has been accomplished, their terminal six to eight glucosyl residues can be removed and used to make additional branches.

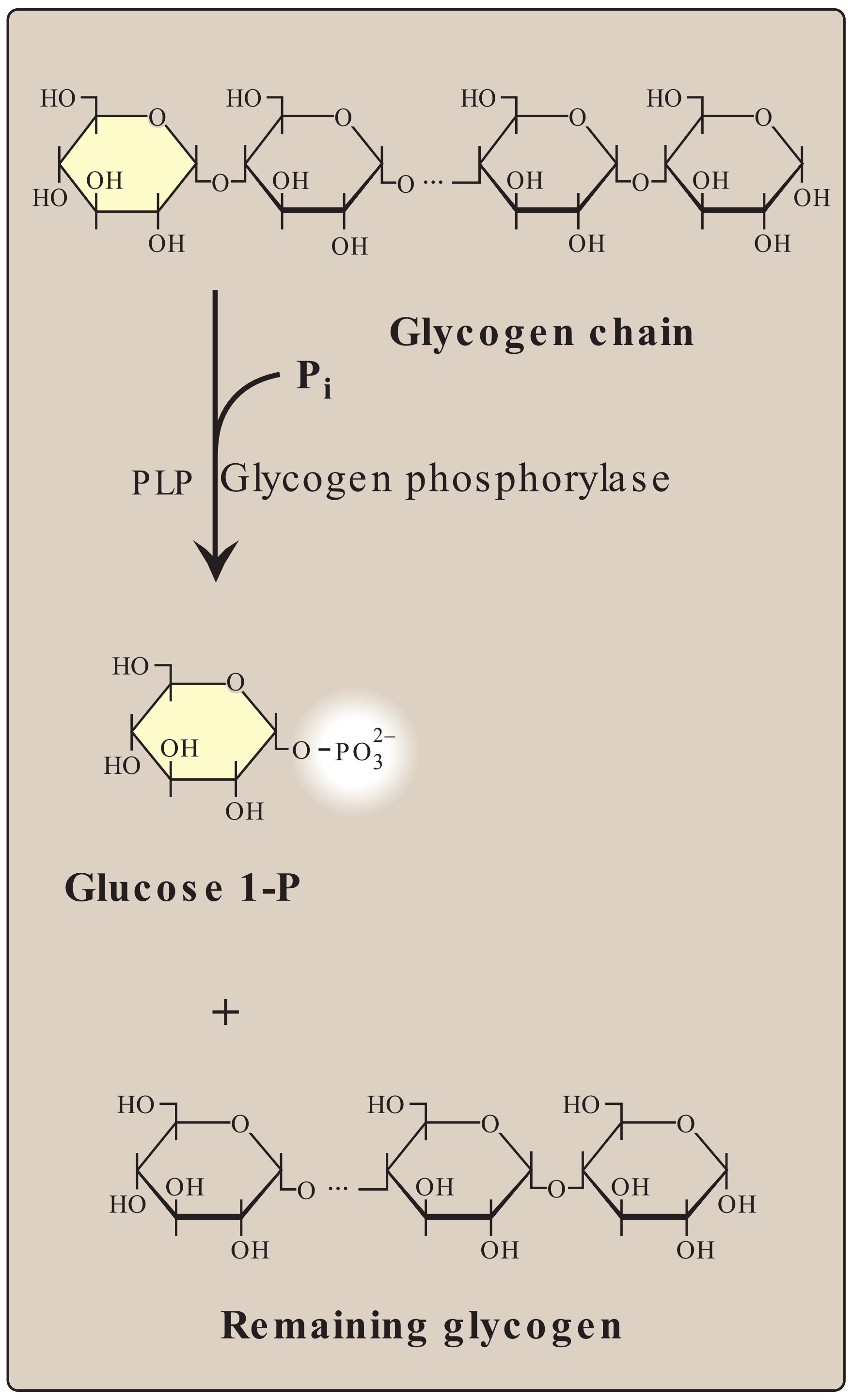


Figure 11.7
Cleavage of an α(1→4)-glycosidic bond. PLP= pyridoxal phosphate; P$_i$ = inorganic phosphate; P = phosphate.

IV. DEGRADATION OF GLYCOGEN (GLYCOGENOLYSIS)

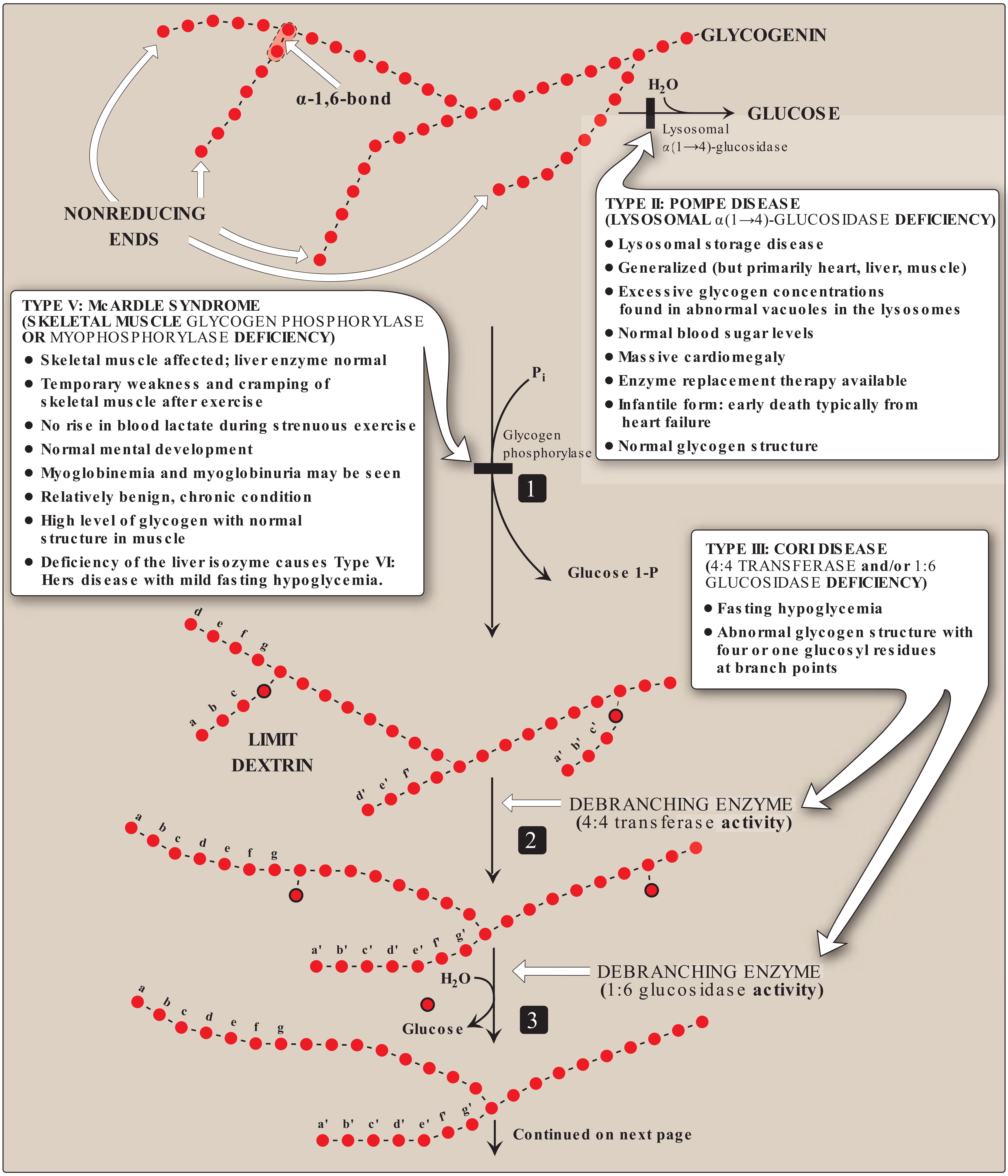
The degradative pathway that mobilizes stored glycogen in liver and skeletal muscle is not a reversal of the synthetic reactions. Instead, a separate set of cytosolic enzymes is required. When glycogen is degraded, the primary product is glucose 1-phosphate, obtained by breaking α(1→4) glycosidic bonds. In addition, free glucose is released from each α(1→6)–linked glucosyl residue (branch point).

A. Shortening of chains

Glycogen phosphorylase sequentially cleaves the α(1→4) glycosidic bonds between the glucosyl residues at the nonreducing ends of the glycogen chains by simple phosphorolysis (producing glucose 1-phosphate) until four glucosyl units remain on each chain before a branch point (Figure 11.7). [Note: Phosphorylase contains a molecule of covalently bound pyridoxal phosphate that is required as a coenzyme.] The resulting structure is called a limit dextrin, and phosphorylase cannot degrade it any further (Figure 11.8).

B. Removal of branches

Branches are removed by the two enzymic activities of a single bifunctional protein, the debranching enzyme (see Figure 11.8). First, oligo-*α*(1→4)→*α*(1→4)-glucantransferase activity removes the outer three of the four glucosyl residues attached at a branch. It next


GLYCOGENIN
α-1,6-bond
NONREDUCING ENDS
H_2O
GLUCOSE
Lysosomal α(1→4)-glucosidase
P_i
Glycogen phosphorylase
1
Glucose 1-P
d e f g
a b c
LIMIT DEXTRIN
a' b' c'
d' e' f'
DEBRANCHING ENZYME (4:4 transferase activity)
2
a b c d e f g
a' b' c' d' e' f' g'
DEBRANCHING ENZYME (1:6 glucosidase activity)
H_2O
Glucose
3
a b c d e f g
a' b' c' d' e' f' g'

Continued on next page

TYPE II: POMPE DISEASE
(LYSOSOMAL α(1→4)-GLUCOSIDASE **DEFICIENCY)**

- Lysosomal storage disease
- Generalized (but primarily heart, liver, muscle)
- Excessive glycogen concentrations found in abnormal vacuoles in the lysosomes
- Normal blood sugar levels
- Massive cardiomegaly
- Enzyme replacement therapy available
- Infantile form: early death typically from heart failure
- Normal glycogen structure

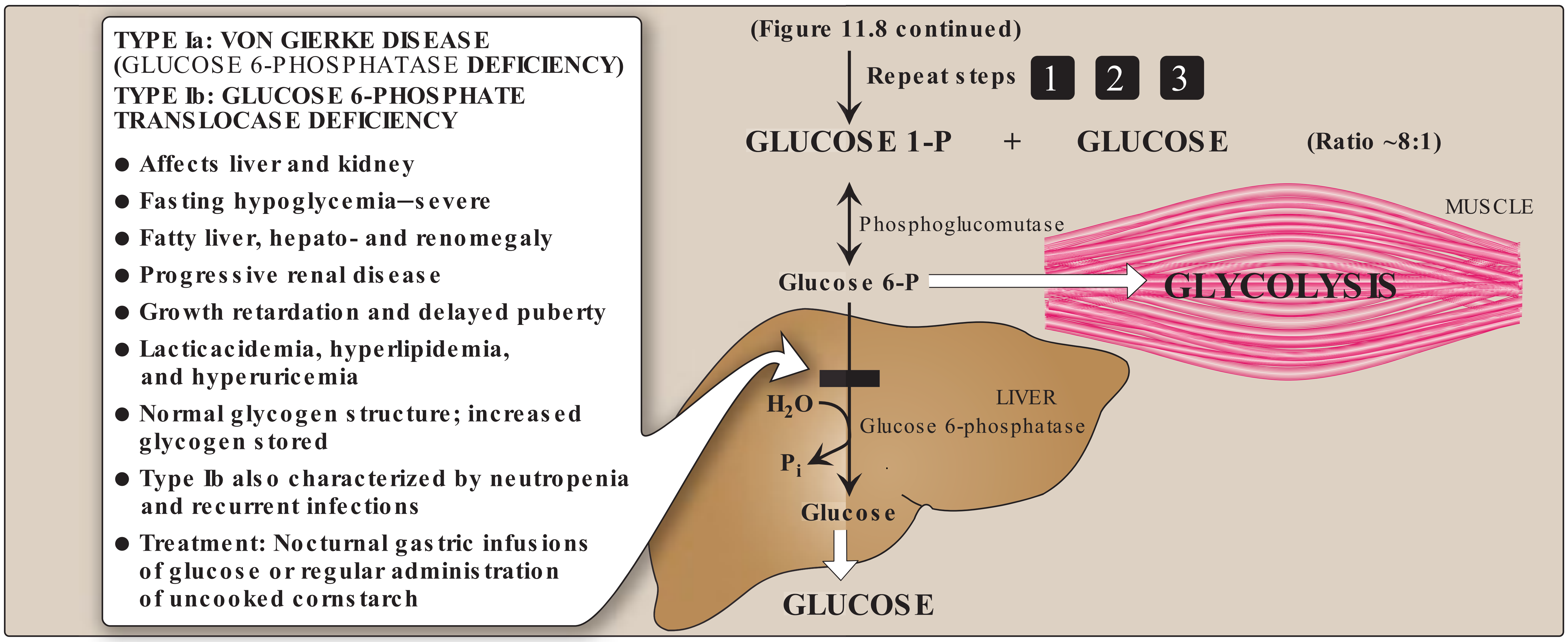
TYPE V: McARDLE SYNDROME
(SKELETAL MUSCLE GLYCOGEN PHOSPHORYLASE
OR MYOPHOSPHORYLASE **DEFICIENCY)**

- Skeletal muscle affected; liver enzyme normal
- Temporary weakness and cramping of skeletal muscle after exercise
- No rise in blood lactate during strenuous exercise
- Normal mental development
- Myoglobinemia and myoglobinuria may be seen
- Relatively benign, chronic condition
- High level of glycogen with normal structure in muscle
- Deficiency of the liver isozyme causes Type VI: Hers disease with mild fasting hypoglycemia.

TYPE III: CORI DISEASE
(4:4 TRANSFERASE **and/or** 1:6 GLUCOSIDASE **DEFICIENCY)**

- Fasting hypoglycemia
- Abnormal glycogen structure with four or one glucosyl residues at branch points

Figure 11.8
Glycogen degradation, showing some of the glycogen storage diseases (GSDs). [Note: A GSD can also be caused by defects in branching enzyme, an enzyme of synthesis, resulting in Type IV: Andersen disease and causing death in early childhood from liver cirrhosis.](Continued on next page.) P_i = inorganic phosphate; P = phosphate.



TYPE Ia: VON GIERKE DISEASE
(GLUCOSE 6-PHOSPHATASE **DEFICIENCY)**
TYPE Ib: GLUCOSE 6-PHOSPHATE TRANSLOCASE DEFICIENCY

- Affects liver and kidney
- Fasting hypoglycemia—severe
- Fatty liver, hepato- and renomegaly
- Progressive renal disease
- Growth retardation and delayed puberty
- Lacticacidemia, hyperlipidemia, and hyperuricemia
- Normal glycogen structure; increased glycogen stored
- Type Ib also characterized by neutropenia and recurrent infections
- Treatment: Nocturnal gastric infusions of glucose or regular administration of uncooked cornstarch

Figure 11.8 (Continued from the previous page.)
Glycogen degradation, showing some of the glycogen storage diseases (GSDs).

transfers them to the nonreducing end of another chain, lengthening it accordingly. Thus, an α(1→4) bond is broken and an α(1→4) bond is made, and the enzyme functions as a 4:4 transferase. Next, the remaining glucose residue attached in an α(1→6) linkage is removed hydrolytically by amylo-*α*(1→6)-glucosidase activity, releasing free glucose. The glucosyl chain is now available again for degradation by glycogen phosphorylase until four glucosyl units in the next branch are reached.

C. Conversion of glucose 1-phosphate to glucose 6-phosphate

Glucose 1-phosphate, produced by glycogen phosphorylase, is converted in the cytosol to glucose 6-phosphate by phosphoglucomutase (see Figure 11.6). In the liver, glucose 6-phosphate is transported into the endoplasmic reticulum (ER) by glucose 6-phosphate translocase. There it is converted to glucose by glucose 6-phosphatase (the same enzyme used in the last step of gluconeogenesis; see p. 121). The glucose then is transported from the ER to the cytosol. Hepatocytes release glycogen-derived glucose into the blood to help maintain blood glucose levels until the gluconeogenic pathway is actively producing glucose. [Note: In the muscle, glucose 6-phosphate cannot be dephosphorylated and sent into the blood because of a lack of glucose 6-phosphatase. Instead, it enters glycolysis, providing energy needed for muscle contraction.]

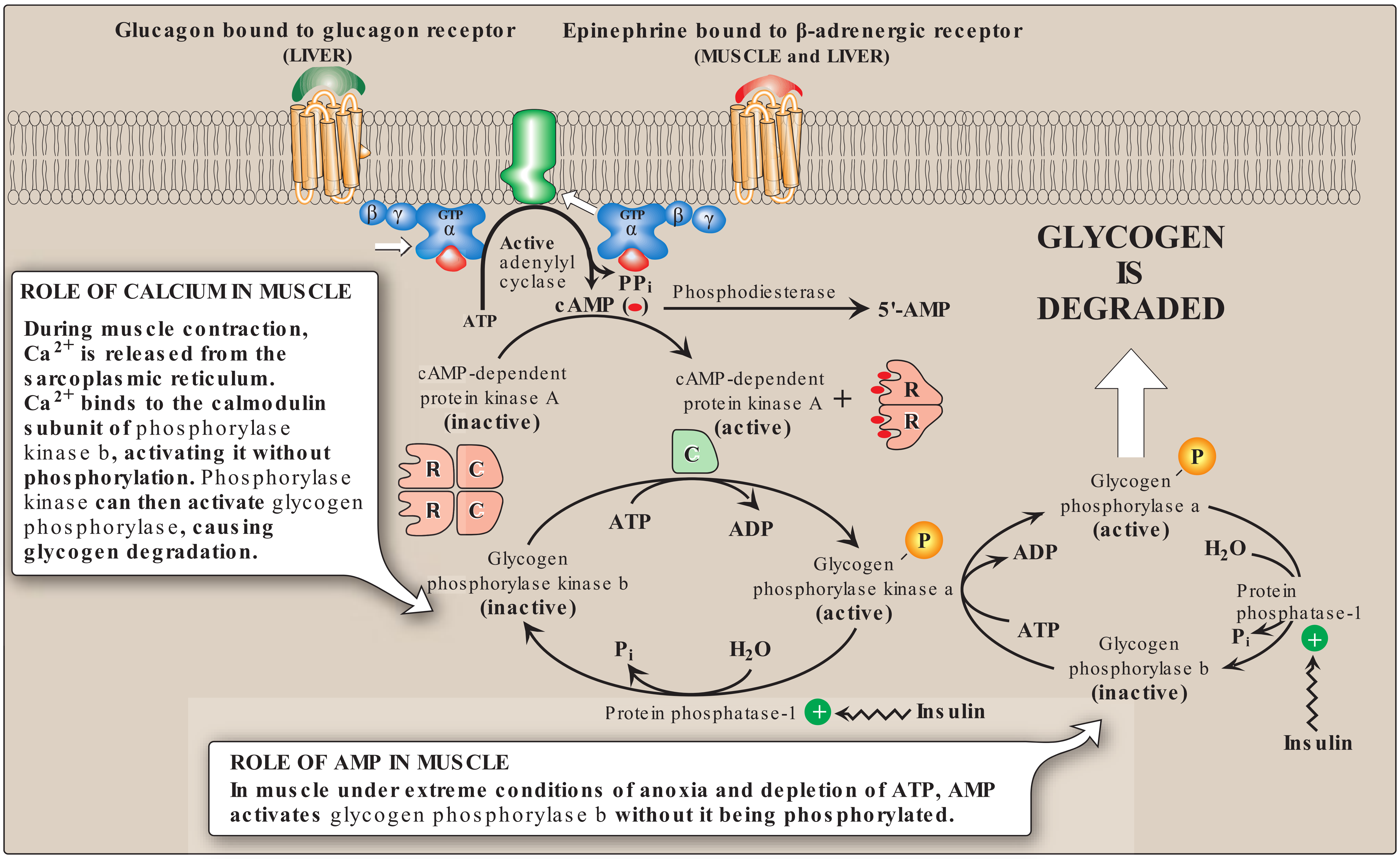
D. Lysosomal degradation of glycogen

A small amount (1%–3%) of glycogen is continuously degraded by the lysosomal enzyme, α(1→4)-glucosidase (acid maltase). The purpose of this pathway is unknown. However, a deficiency of this enzyme causes accumulation of glycogen in vacuoles in the lysosomes, resulting in the serious glycogen storage disease (GSD) Type II: Pompe disease (see Figure 11.8). [Note: Type II: Pompe disease is the only GSD that is a lysosomal storage disease.]

Lysosomal storage diseases are genetic disorders characterized by the accumulation of abnormal amounts of carbohydrates or lipids primarily due to their decreased lysosomal degradation.

V. REGULATION OF GLYCOGENESIS AND GLYCOGENOLYSIS

Because of the importance of maintaining blood glucose levels, the synthesis and degradation of its glycogen storage form are tightly regulated. In the liver, glycogenesis accelerates during periods when the body has been well fed, whereas glycogenolysis accelerates during periods of fasting. In skeletal muscle, glycogenolysis occurs during active exercise, and glycogenesis begins as soon as the muscle is again at rest. Regulation of glycogen synthesis and degradation is accomplished on two levels. First, glycogen synthase and glycogen phosphorylase are hormonally regulated (by phosphorylation/dephosphorylation) to meet the needs of the body as a whole. [Note: Phosphorylation of glycogen phosphorylase is catalyzed by glycogen phosphorylase kinase (see p. 132).] Second, these same enzymes are allosterically regulated (by effector molecules) to meet the needs of a particular tissue.


Glucagon bound to glucagon receptor
(LIVER)
Epinephrine bound to β-adrenergic receptor
(MUSCLE and LIVER)
β
γ
GTP
α
Active adenylyl cyclase
ATP
PPi
cAMP
Phosphodiesterase
5'-AMP
GLYCOGEN IS DEGRADED
cAMP-dependent protein kinase A
(inactive)
cAMP-dependent protein kinase A
(active)
+
R
C
ATP
ADP
P
Glycogen phosphorylase kinase b
(inactive)
Glycogen phosphorylase kinase a
(active)
Pi
H2O
Protein phosphatase-1
+
Insulin
Glycogen phosphorylase a
(active)
ADP
ATP
H2O
Protein phosphatase-1
Pi
Glycogen phosphorylase b
(inactive)
Insulin

ROLE OF CALCIUM IN MUSCLE

During muscle contraction, Ca^{2+} is released from the sarcoplasmic reticulum. Ca^{2+} binds to the calmodulin subunit of phosphorylase kinase b**, activating it without phosphorylation.** Phosphorylase kinase **can then activate** glycogen phosphorylase**, causing glycogen degradation.**

ROLE OF AMP IN MUSCLE

In muscle under extreme conditions of anoxia and depletion of ATP, AMP activates glycogen phosphorylase b **without it being phosphorylated.**

Figure 11.9
Stimulation and inhibition of glycogen degradation. AMP = adenosine monophosphate; cAMP = cyclic AMP; GTP = guanosine triphosphate; P = phosphate; PP_i = pyrophosphate; R = regulatory subunit; C = catalytic subunit.

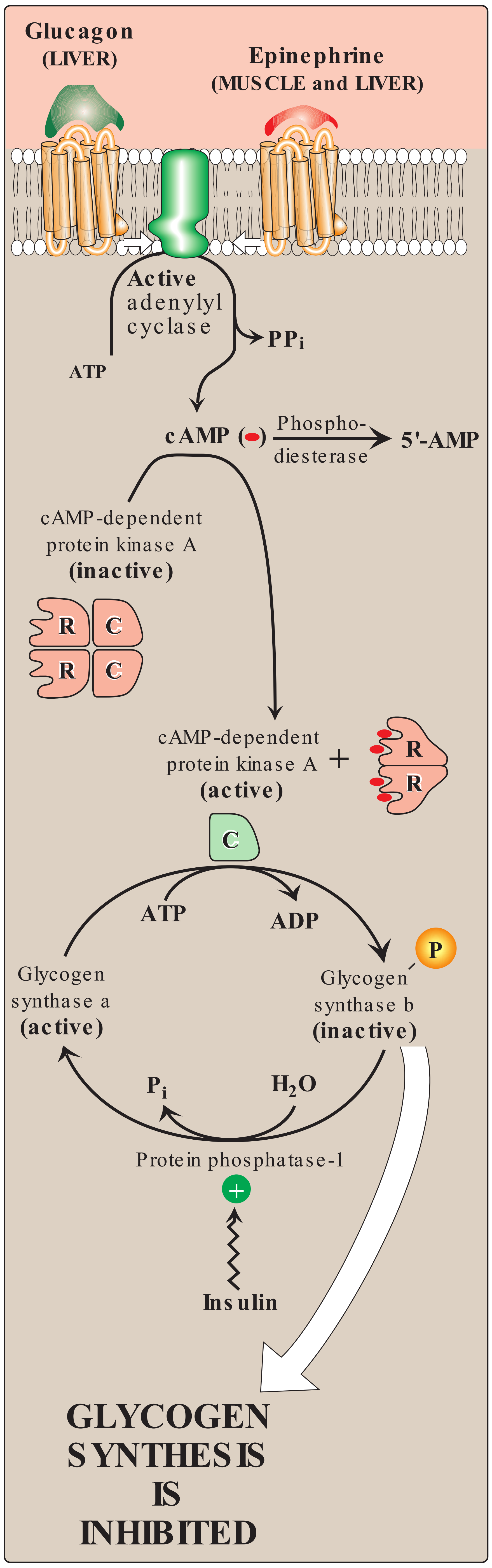


Figure 11.10
Hormonal regulation of glycogen synthesis. [Note: In contrast to glycogen phosphorylase, glycogen synthase is inactivated by phosphorylation.] cAMP = cyclic adenosine monophosphate; P = phosphate; PP$_i$ = pyrophosphate; R = regulatory subunit; C = catalytic subunit.

A. Activation of glycogen degradation

The binding of hormones, such as glucagon or epinephrine, to plasma membrane G protein–coupled receptors (GPCRs) signals the need for glycogen to be degraded, either to elevate blood glucose levels or to provide energy for exercising muscle.

1. **Activation of protein kinase A:** Binding of glucagon or epinephrine to their specific hepatocyte GPCR, or of epinephrine to a specific myocyte GPCR, results in the G protein–mediated activation of adenylyl cyclase. This enzyme catalyzes the synthesis of cyclic adenosine monophosphate (cAMP), which activates cAMP-dependent protein kinase A (PKA), as described on page 95. PKA is a tetramer, having two regulatory subunits (R) and two catalytic subunits (C). cAMP binds to the regulatory subunits, releasing individual catalytic subunits that are active (Figure 11.9). PKA then phosphorylates several enzymes of glycogen metabolism, affecting their activity. [Note: When cAMP is removed, the inactive tetramer, R_2C_2, is again formed.]

2. **Activation of phosphorylase kinase:** Phosphorylase kinase exists in two forms: an inactive "b" form and an active "a" form. Active PKA phosphorylates the inactive "b" form of phosphorylase kinase, producing the active "a" form (see Figure 11.9).

3. **Activation of glycogen phosphorylase:** Glycogen phosphorylase also exists in two forms: the dephosphorylated, inactive "b" form and the phosphorylated, active "a" form. Active phosphorylase kinase is the only enzyme that phosphorylates glycogen phosphorylase b to its active "a" form, which then begins glycogenolysis (see Figure 11.9).

4. **Summary of the regulation of glycogen degradation:** The cascade of reactions listed above results in glycogenolysis. The large number of sequential steps serves to amplify the effect of the hormonal signal (that is, a few hormone molecules binding to their receptors results in a number of PKA molecules being activated that can each activate many phosphorylase kinase molecules). This causes the production of many active glycogen phosphorylase a molecules that can degrade glycogen.

5. **Maintenance of the phosphorylated state:** The phosphate groups added to phosphorylase kinase and phosphorylase in response to cAMP are maintained because the enzyme that hydrolytically removes the phosphate, protein phosphatase-1 (PP1), is inactivated by inhibitor proteins that are also phosphorylated and activated in response to cAMP (see Figure 11.9). [Note: PP1 is activated by a signal cascade initiated by insulin (see p. 311). Insulin also activates the phosphodiesterase that degrades cAMP, and, thus, insulin opposes the effects of glucagon and epinephrine.]

B. Inhibition of glycogen synthesis

The regulated enzyme in glycogenesis is glycogen synthase. It also exists in two forms, the active "a" form and the inactive "b" form. However, for glycogen synthase, in contrast to phosphorylase kinase and phosphorylase, the active form is dephosphorylated, whereas the inactive form is phosphorylated (Figure 11.10). Glycogen synthase a is converted to the inactive "b" form by phosphorylation at several sites on the enzyme, with the level of inactivation proportional to its degree of phosphorylation. Phosphorylation is catalyzed by several different protein kinases that are regulated by cAMP or other signaling mechanisms (see C. below). Glycogen synthase b can be reconverted to the "a" form by PP1. Figure 11.11 summarizes the covalent regulation of glycogen metabolism.

C. Allosteric regulation of glycogen synthesis and degradation

In addition to hormonal signals, glycogen synthase and glycogen phosphorylase respond to the levels of metabolites and energy needs of the cell. Glycogenesis is stimulated when substrate availability and energy levels are high, whereas glycogenolysis is increased when glucose and energy levels are low. This allosteric regulation allows a rapid response to the needs of a cell and can override the effects of hormone-mediated covalent regulation.

1. **Regulation of glycogen synthesis and degradation in the well-fed state:**

   In the well-fed state, glycogen synthase b in both liver and muscle is allosterically activated by glucose 6-phosphate, which is present in elevated concentrations (Figure 11.12). In contrast, glycogen phosphorylase a is allosterically inhibited by glucose 6-phosphate, as well as by ATP, a high-energy signal in the cell. [Note: In liver, but not muscle, nonphosphorylated glucose is also an allosteric inhibitor of glycogen phosphorylase a, making it a better substrate for PP1.]

2. **Activation of glycogen degradation by calcium:** Ca^{2+} is released into the cytoplasm in muscle in response to neural stimulation and in liver in response to epinephrine binding to α_1-adrenergic receptors. The Ca^{2+} binds to calmodulin (CaM), the most widely distributed member of a family of small, calcium-binding proteins. The binding of four molecules of Ca^{2+} to CaM triggers a conformational change such that the activated Ca^{2+}–CaM complex binds to and activates protein molecules, often enzymes, that are inactive in the absence of this complex (see Figure 11.13). Thus, CaM functions as an essential subunit of many complex proteins. One such protein is the tetrameric phosphorylase kinase, whose b form is activated by the binding of Ca^{2+} to its δ subunit (CaM) without the need for the kinase to be phosphorylated by PKA. [Note: Epinephrine at β-adrenergic receptors signals through a rise in cAMP, not Ca^{2+} (see p. 131).]

   a. **Calcium activation of muscle phosphorylase kinase:** During muscle contraction, there is a rapid and urgent need for ATP. This energy is supplied by the degradation of muscle glycogen to glucose, which can then enter glycolysis. Nerve impulses cause membrane depolarization, which promotes Ca^{2+} release from the sarcoplasmic reticulum into the sarcoplasm of myocytes. The Ca^{2+} binds the CaM subunit, and the complex activates muscle phosphorylase kinase b (see Figure 11.9).

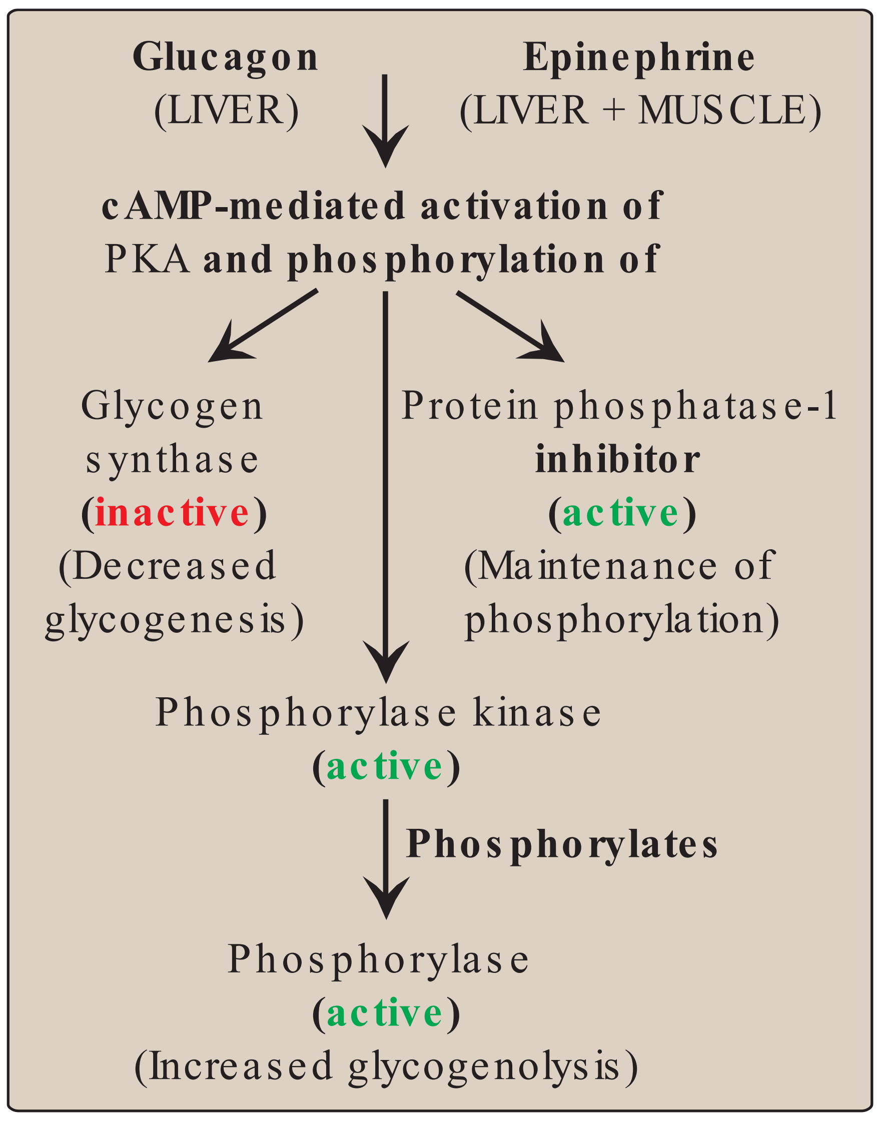

Glucagon
(LIVER)
Epinephrine
(LIVER + MUSCLE)
cAMP-mediated activation of
PKA and phosphorylation of
Glycogen synthase
(inactive)
(Decreased glycogenesis)
Protein phosphatase-1 inhibitor
(active)
(Maintenance of phosphorylation)
Phosphorylase kinase
(active)
Phosphorylates
Phosphorylase
(active)
(Increased glycogenolysis)

Figure 11.11
Summary of the hormone-mediated covalent regulation of glycogen metabolism. cAMP = cyclic AMP; PKA = protein kinase A.

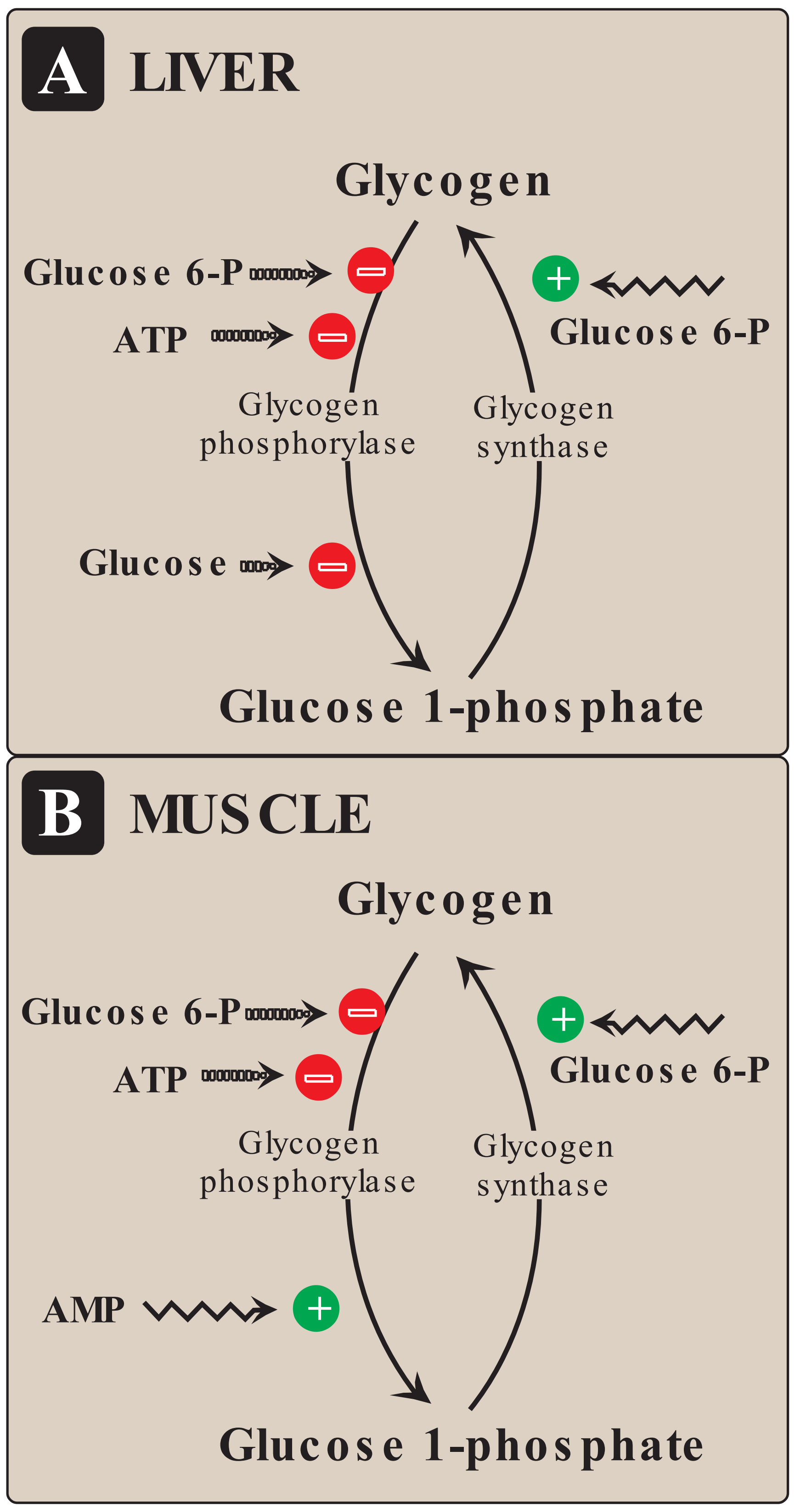
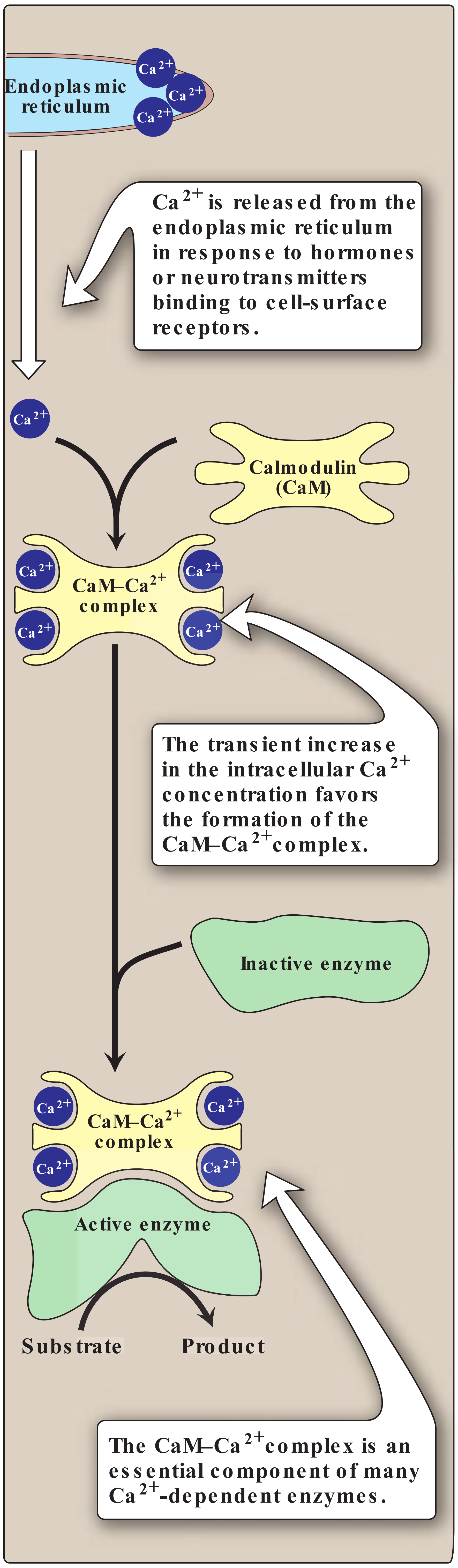

A LIVER
Glycogen
Glucose 6-P
ATP
Glycogen phosphorylase
Glucose
Glucose 6-P
Glycogen synthase
Glucose 1-phosphate
B MUSCLE
Glycogen
Glucose 6-P
ATP
Glycogen phosphorylase
AMP
Glucose 6-P
Glycogen synthase
Glucose 1-phosphate

Figure 11.12
Allosteric regulation of glycogen synthesis and degradation.
A. Liver. B. Muscle. P = phosphate.



Ca^{2+} is released from the endoplasmic reticulum in response to hormones or neurotransmitters binding to cell-surface receptors.

The transient increase in the intracellular Ca^{2+} concentration favors the formation of the $CaM–Ca^{2+}$ complex.

The $CaM–Ca^{2+}$ complex is an essential component of many Ca^{2+}-dependent enzymes.

Figure 11.13
Calmodulin mediates many effects of intracellular Ca^{2+}.

b. Calcium activation of liver phosphorylase kinase: During physiologic stress, epinephrine is released from the adrenal medulla and signals the need for blood glucose. This glucose initially comes from hepatic glycogenolysis. Binding of epinephrine to hepatocyte α-adrenergic GPCRs activates a phospholipid-dependent cascade (see p. 205) that results in movement of Ca^{2+} from the ER into the cytoplasm. A Ca^{2+}–CaM complex forms and activates hepatic phosphorylase kinase b. [Note: The released Ca^{2+} also helps to activate protein kinase C that can phosphorylate (therefore, inactivate) glycogen synthase a.]

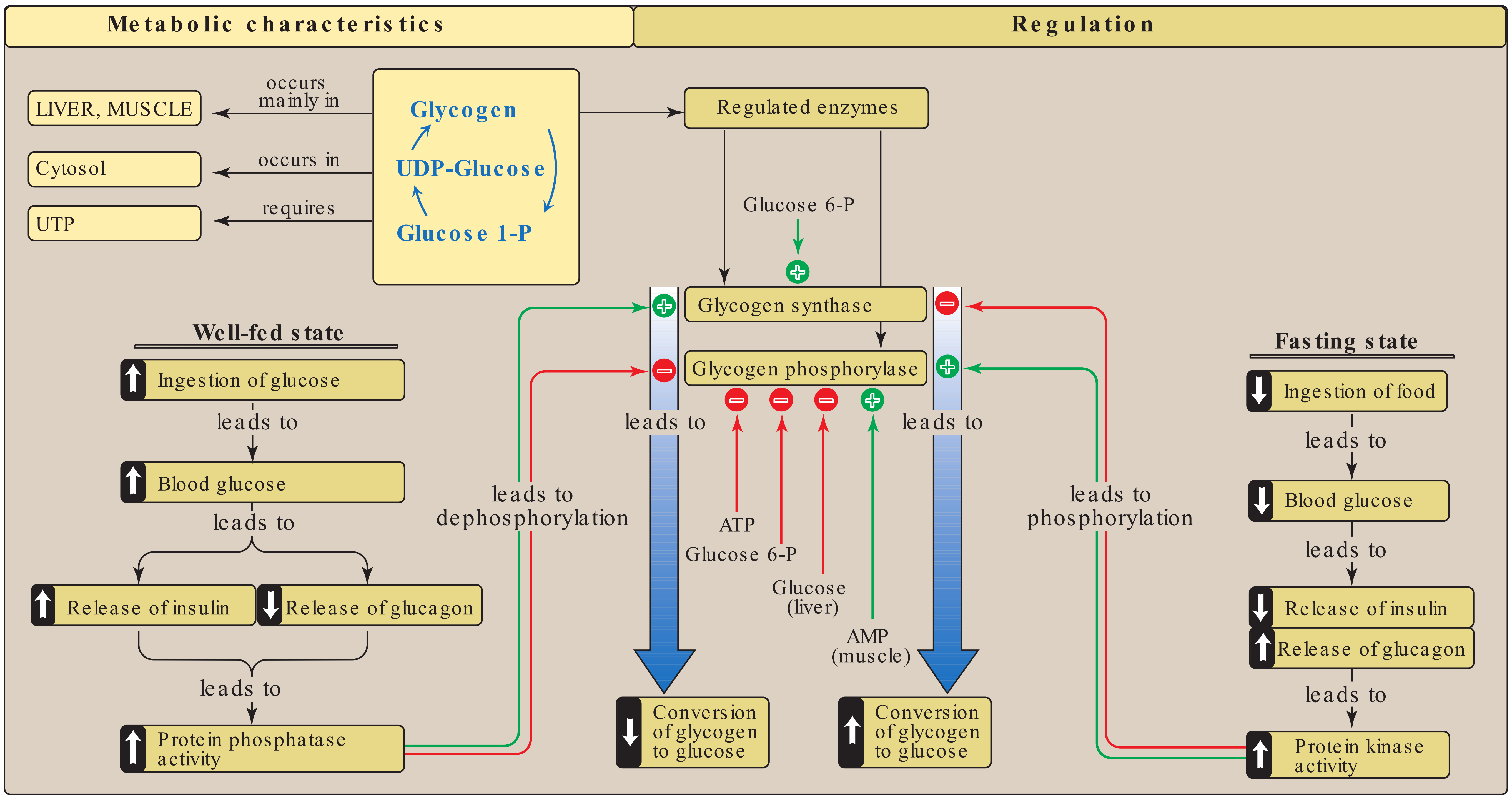
3. Activation of glycogen degradation in muscle: Muscle glycogen phosphorylase is active in the presence of the high adenosine monophosphate (AMP) concentrations that occur under extreme conditions of anoxia and ATP depletion. AMP binds to glycogen phosphorylase b, causing its activation without phosphorylation (see Figure 11.9). [Note: Recall that AMP also activates phosphofructokinase-1 of glycolysis (see p. 99), allowing glucose from glycogenolysis to be oxidized.]

VI. GLYCOGEN STORAGE DISEASES

These are a group of genetic diseases that are caused by defects in enzymes required for glycogen degradation or, more rarely, glycogen synthesis. They result either in formation of glycogen that has an abnormal structure or in the accumulation of excessive amounts of normal glycogen in specific tissues as a result of impaired degradation. A particular enzyme may be defective in a single tissue, such as liver (resulting in hypoglycemia) or muscle (causing muscle weakness), or the defect may be more generalized, affecting a variety of tissues. The severity of the GSDs ranges from fatal in early childhood to mild disorders that are not life threatening. Some of the more prevalent GSDs are illustrated in Figure 11.8. [Note: Only one GSD is lysosomal because glycogen metabolism occurs primarily in the cytosol.]

VII. CHAPTER SUMMARY

The **main stores** of glycogen in the body are found in **skeletal muscle**, where they serve as a **fuel reserve** for the synthesis of ATP during **muscle contraction**, and in the **liver**, where they are used to **maintain the blood glucose** concentration, particularly during the **early stages of a fast**. Glycogen is a **highly branched** polymer of **α-D-glucose**. The primary glycosidic bond is an **α(1→4) linkage**. After about eight to ten glucosyl residues, there is a **branch** containing an **α(1→6) linkage**. **Uridine diphosphate (UDP)-glucose**, the **building block** of glycogen, is synthesized from **glucose 1-phosphate** and **UTP** by UDP-glucose pyrophosphorylase (Figure 11.14). Glucose from UDP-glucose is transferred to the nonreducing ends of glycogen chains by primer-requiring glycogen synthase, which makes **α(1→4) linkages**. The primer is made by glycogenin. **Branches** are formed by amylo-α(1→4)→α(1→6)-transglucosidase (common name, glucosyl 4:6 transferase), which transfers a set of six to eight glucosyl residues from the nonreducing end of the glycogen chain (**breaking** an **α(1→4) linkage**), and attaches it with an **α(1→6) linkage** to another residue in the chain. Pyridoxal phosphate–requiring glycogen phosphorylase cleaves the **α(1→4) bonds** between glucosyl residues at the **nonreducing ends** of the glycogen chains, producing **glucose 1-phosphate**. This sequential degradation continues until four glucosyl units remain before a branch point. The resulting structure is called a **limit dextrin** that is degraded by the bifunctional debranching enzyme. Oligo-α(1→4)→α(1→4)-glucantransferase (common name, glucosyl 4:4 transferase) removes the outer three of the four glucosyl residues at a branch and transfers them to the nonreducing end of another chain, where they can be converted to glucose 1-phosphate by glycogen phosphorylase. The remaining single glucose residue attached in an **α(1→6) linkage** is removed hydrolytically by the amylo-(1→6) glucosidase activity of debranching enzyme, releasing **free glucose**. **Glucose 1-phosphate** is converted to **glucose 6-phosphate** by phosphoglucomutase. In the **muscle**, glucose 6-phosphate enters glycolysis. In the **liver**, the phosphate is removed by glucose 6-phosphatase, releasing **free glucose** that can be used to maintain blood glucose levels at the beginning of a fast. A **deficiency** of the phosphatase causes **glycogen storage disease Type 1a** (**Von Gierke disease**). This disease results in an inability of the liver to provide free glucose to the body during a fast. It affects both glycogen degradation and gluconeogenesis. Glycogen synthesis and degradation are reciprocally regulated to meet whole-body needs by the same hormonal signals (namely, an **elevated insulin** level results in overall **increased glycogenesis** and **decreased glycogenolysis**, whereas an **elevated glucagon**, or **epinephrine**, level causes **increased glycogenolysis** and **decreased glycogenesis**). Key enzymes are phosphorylated by a family of protein kinases, some of which are **cyclic adenosine monophosphate dependent** (a compound increased by glucagon and epinephrine). Phosphate groups are removed by protein phosphatase-1 (active when its inhibitor is inactive in response to elevated insulin levels). Glycogen synthase**,** phosphorylase kinase, and phosphorylase are also **allosterically regulated** to meet tissues needs. In the well-fed state, glycogen synthase is **activated** by **glucose 6-phosphate**, but glycogen phosphorylase is inhibited by **glucose 6-phosphate** as well as by **ATP**. In the liver, glucose also serves an an allosteric inhibitor of glycogen phosphorylase. The **Ca^{2+}** released from the endoplasmic reticulum in muscle during exercise and in liver in response to epinephrine **activates** phosphorylase kinase by binding to the enzyme's **calmodulin** subunit. This allows the enzyme to activate glycogen phosphorylase, thereby causing glycogen degradation. **AMP** activates glycogen phosphorylase in muscle.



Metabolic characteristics
Regulation
LIVER, MUSCLE
occurs mainly in
Cytosol
occurs in
UTP
requires
Glycogen
UDP-Glucose
Glucose 1-P
Regulated enzymes
Glucose 6-P
Glycogen synthase
Glycogen phosphorylase
leads to
leads to
Well-fed state
Ingestion of glucose
leads to
Blood glucose
leads to
Release of insulin
Release of glucagon
leads to
Protein phosphatase activity
leads to dephosphorylation
ATP
Glucose 6-P
Glucose (liver)
AMP (muscle)
Conversion of glycogen to glucose
Conversion of glycogen to glucose
leads to phosphorylation
Fasting state
Ingestion of food
leads to
Blood glucose
leads to
Release of insulin
Release of glucagon
leads to
Protein kinase activity

Figure 11.14
Key concept map for glycogen metabolism in the liver. [Note: Glycogen phosphorylase is phosphorylated by phosphorylase kinase, the "b" form of which can be activated by calcium.] UDP = uridine diphosphate; UTP = uridine triphosphate; P = phosphate.

Metabolism of Monosaccharides and Disaccharides

12

I. OVERVIEW

Glucose is the most common monosaccharide consumed by humans, and its metabolism has already been discussed. However, two other monosaccharides, fructose and galactose, occur in significant amounts in the diet (primarily in disaccharides) and make important contributions to energy metabolism. In addition, galactose is an important component of structural carbohydrates. Figure 12.1 shows the metabolism of fructose and galactose as part of the essential pathways of energy metabolism.

II. FRUCTOSE METABOLISM

About 10% of the calories comprising the Western diet are supplied by fructose (approximately 55 g/day). The major source of fructose is the disaccharide sucrose, which, when cleaved in the intestine, releases equimolar amounts of fructose and glucose. Fructose is also found as a free monosaccharide in many fruits, in honey, and in high-fructose corn syrup (typically, 55% fructose/45% glucose), which is used to sweeten soft drinks and many foods. Fructose transport into cells is not insulin dependent (unlike that of glucose into certain tissues; see p. 97), and, in contrast to glucose, fructose does not promote the secretion of insulin.

A. Phosphorylation of fructose

For fructose to enter the pathways of intermediary metabolism, it must first be phosphorylated (Figure 12.2). This can be accomplished by either hexokinase or fructokinase. Hexokinase phosphorylates glucose in most cells of the body (see p. 98), and several additional hexoses can serve as substrates for this enzyme. However, it has a low affinity (that is, a high Michaelis constant [K_m]; see p. 59) for fructose. Therefore, unless the intracellular concentration of fructose becomes unusually high, the normal presence of saturating concentrations of glucose means that little fructose is phosphorylated by hexokinase. Fructokinase provides the primary mechanism for fructose phosphorylation (see Figure 12.2). The enzyme has a low K_m for fructose and a high V_{max} (or, maximal velocity; see p. 59). It is found in the liver (which processes most of the dietary fructose), kidney, and the small intestinal mucosa and converts fructose to fructose 1-phosphate, using adenosine triphosphate (ATP) as the phosphate donor. [Note: These three tissues also contain aldolase B, discussed in section B.]

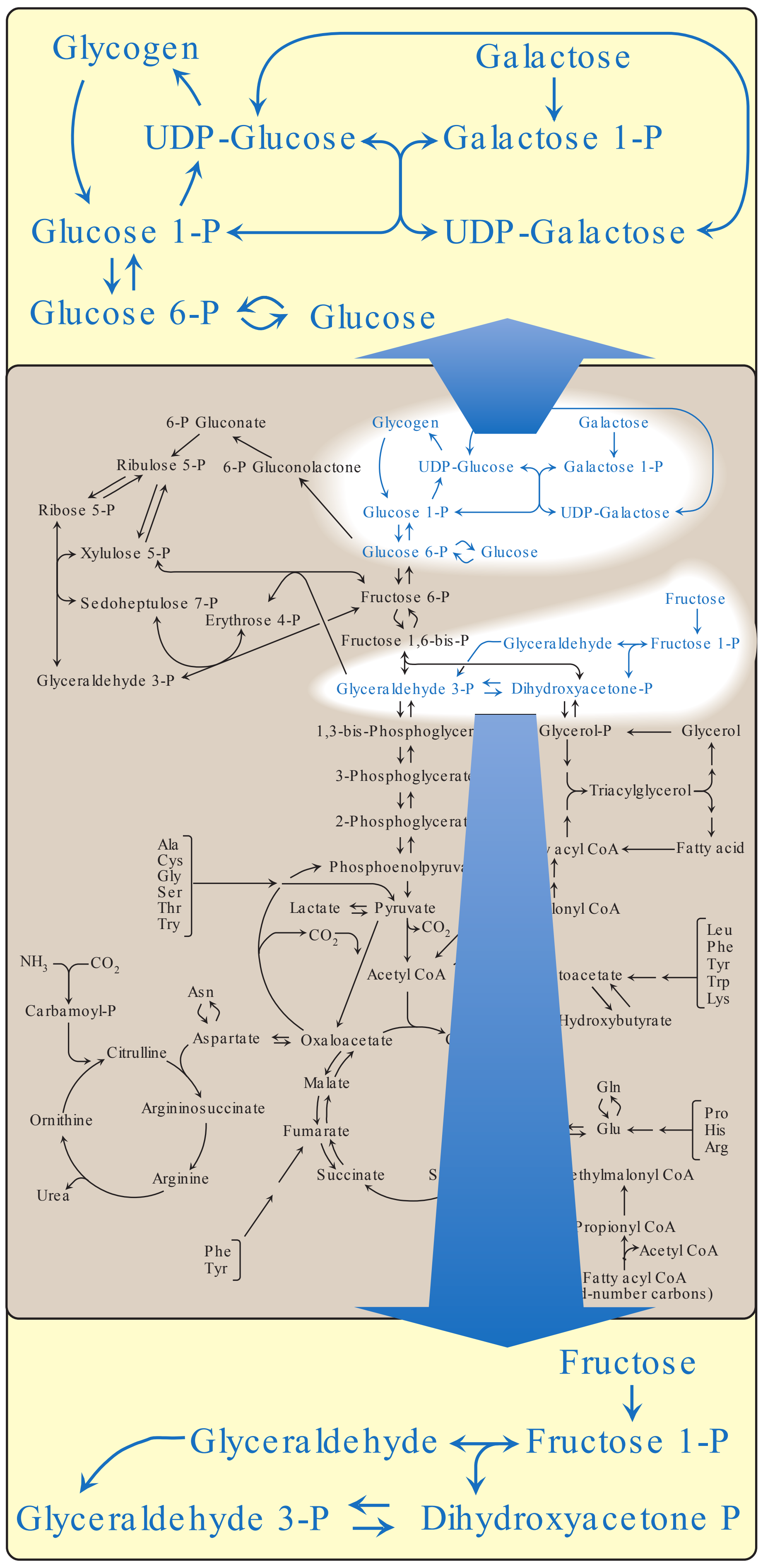
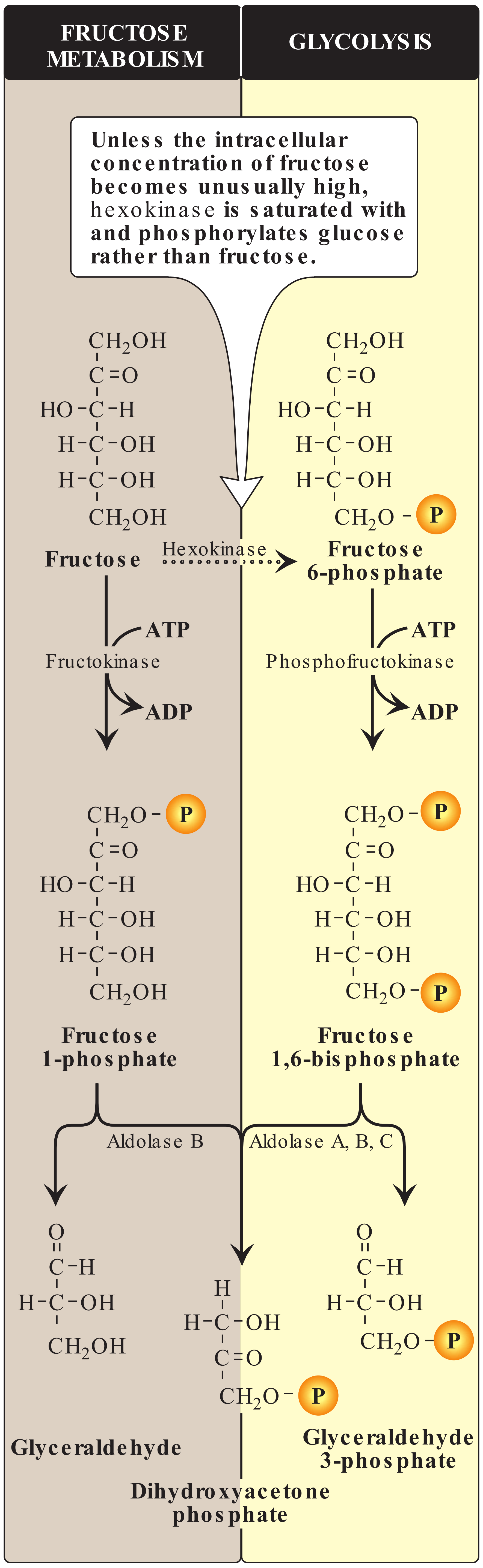


Figure 12.1
Galactose and fructose metabolism as part of the essential pathways of energy metabolism (see Figure 8.2, p. 92, for a more detailed view of the overall reactions of metabolism). UDP = uridine diphosphate; P = phosphate.



Unless the intracellular concentration of fructose becomes unusually high, hexokinase **is saturated with and phosphorylates glucose rather than fructose.**

Figure 12.2
Phosphorylation products of fructose and their cleavage. P = phosphate; ADP = adenosine diphosphate.

B. Cleavage of fructose 1-phosphate

Fructose 1-phosphate is not phosphorylated to fructose 1,6-bisphosphate as is fructose 6-phosphate (see p. 99) but is cleaved by aldolase B (also called fructose 1-phosphate aldolase) to dihydroxyacetone phosphate (DHAP) and glyceraldehyde. [Note: Humans express three aldolases, A, B and C, the products of three different genes. Aldolase A (found in most tissues), aldolase B (in liver, kidney, and small intestine), and aldolase C (in brain) all cleave fructose 1,6-bisphosphate produced during glycolysis to DHAP and glyceraldehyde 3-phosphate (see p. 100), but only aldolase B cleaves fructose 1-phosphate.] DHAP can directly enter glycolysis or gluconeogenesis, whereas glyceraldehyde can be metabolized by a number of pathways, as illustrated in Figure 12.3.

C. Kinetics of fructose metabolism

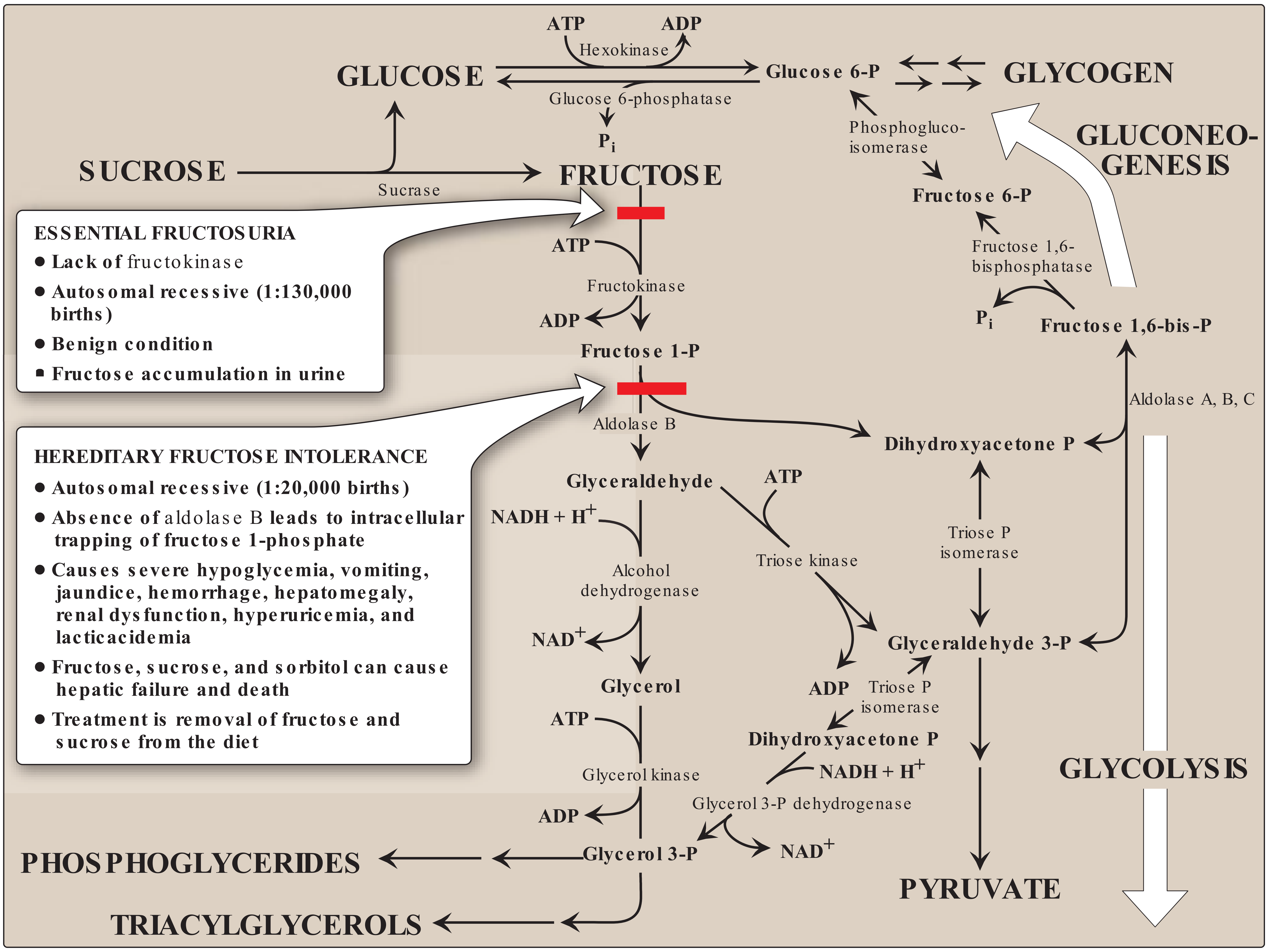
The rate of fructose metabolism is more rapid than that of glucose because the trioses formed from fructose 1-phosphate bypass phosphofructokinase-1, the major rate-limiting step in glycolysis (see p. 99).

D. Disorders of fructose metabolism

A deficiency of one of the key enzymes required for the entry of fructose into metabolic pathways can result in either a benign condition as a result of fructokinase deficiency (essential fructosuria) or a severe disturbance of liver and kidney metabolism as a result of aldolase B deficiency (hereditary fructose intolerance [HFI]), which is estimated to occur in 1:20,000 live births (see Figure 12.3). The first symptoms of HFI appear when a baby is weaned from milk (see p. 142) and begins to be fed food containing sucrose or fructose. Fructose 1-phosphate accumulates, resulting in a drop in the level of inorganic phosphate (P_i) and, therefore, of ATP production. As ATP falls, adenosine monophosphate (AMP) rises. The AMP is degraded, causing hyperuricemia (and lactic acidosis; see p. 299). The decreased availability of hepatic ATP affects gluconeogenesis (causing hypoglycemia with vomiting) and protein synthesis (causing a decrease in blood clotting factors and other essential proteins). Kidney function may also be affected. [Note: The drop in P_i also inhibits glycogenolysis (see p. 128).] Diagnosis of HFI can be made on the basis of fructose in the urine, enzyme assay using liver cells, or by DNA-based testing (see Chapter 33). Aldolase B deficiency is part of the newborn screening panel. With HFI, sucrose, as well as fructose, must be removed from the diet to prevent liver failure and possible death. Individuals with HFI display an aversion to sweets and, consequently, have an absence of dental caries.

E. Conversion of mannose to fructose 6-phosphate

Mannose, the C-2 epimer of glucose (see p. 84), is an important component of glycoproteins (see p. 166). Hexokinase phosphorylates mannose, producing mannose 6-phosphate, which, in turn, is reversibly isomerized to fructose 6-phosphate by phosphomannose isomerase. [Note: There is little mannose in dietary carbohydrates. Most intracellular mannose is synthesized from fructose or is preexisting mannose produced by the degradation of structural carbohydrates and salvaged by hexokinase.]


ATP
ADP
Hexokinase
GLUCOSE
Glucose 6-P
GLYCOGEN
Glucose 6-phosphatase
P_i
Phosphogluco-isomerase
GLUCONEO-GENESIS
SUCROSE
Sucrase
FRUCTOSE
Fructose 6-P
ATP
Fructose 1,6-bisphosphatase
Fructokinase
ADP
P_i
Fructose 1,6-bis-P
Fructose 1-P
Aldolase A, B, C
Aldolase B
Dihydroxyacetone P
Glyceraldehyde
ATP
NADH + H^+
Triose kinase
Triose P isomerase
Alcohol dehydrogenase
NAD$^+$
Glyceraldehyde 3-P
Glycerol
ADP
Triose P isomerase
ATP
Dihydroxyacetone P
NADH + H^+
GLYCOLYSIS
Glycerol kinase
Glycerol 3-P dehydrogenase
ADP
PHOSPHOGLYCERIDES
Glycerol 3-P
NAD$^+$
PYRUVATE
TRIACYLGLYCEROLS

ESSENTIAL FRUCTOSURIA

- **Lack of** fructokinase
- **Autosomal recessive (1:130,000 births)**
- **Benign condition**
- **Fructose accumulation in urine**

HEREDITARY FRUCTOSE INTOLERANCE

- **Autosomal recessive (1:20,000 births)**
- **Absence of** aldolase B **leads to intracellular trapping of fructose 1-phosphate**
- **Causes severe hypoglycemia, vomiting, jaundice, hemorrhage, hepatomegaly, renal dysfunction, hyperuricemia, and lacticacidemia**
- **Fructose, sucrose, and sorbitol can cause hepatic failure and death**
- **Treatment is removal of fructose and sucrose from the diet**

Figure 12.3
Summary of fructose metabolism. P = phosphate; P_i = inorganic phosphate; NAD(H) = nicotinamide adenine dinucleotide; ADP = adenosine diphosphate.

F. Conversion of glucose to fructose via sorbitol

Most sugars are rapidly phosphorylated following their entry into cells. Therefore, they are trapped within the cells, because organic phosphates cannot freely cross membranes without specific transporters. An alternate mechanism for metabolizing a monosaccharide is to convert it to a polyol (sugar alcohol) by the reduction of an aldehyde group, thereby producing an additional hydroxyl group.

1. **Synthesis of sorbitol:** Aldose reductase reduces glucose, producing sorbitol (glucitol; Figure 12.4). This enzyme is found in many tissues, including the lens, retina, Schwann cells of peripheral nerves, liver, kidney, placenta, red blood cells, and cells of the ovaries and seminal vesicles. In cells of the liver, ovaries, and seminal vesicles, there is a second enzyme, sorbitol dehydrogenase, which can oxidize the sorbitol to produce fructose (see Figure 12.4). The two-reaction pathway from glucose to fructose in the seminal vesicles benefits sperm cells, which use fructose as a major carbohydrate energy source. The pathway from sorbitol to fructose in the liver

provides a mechanism by which any available sorbitol is converted into a substrate that can enter glycolysis or gluconeogenesis.

2. **Effect of hyperglycemia on sorbitol metabolism:** Because insulin is not required for the entry of glucose into the cells listed in the previous paragraph, large amounts of glucose may enter these cells during times of hyperglycemia (for example, in uncontrolled diabetes). Elevated intracellular glucose concentrations and an adequate supply of reduced nicotinamide adenine dinucleotide phosphate (NADPH) cause aldose reductase to produce a significant increase in the amount of sorbitol, which cannot pass efficiently through cell membranes and, in turn, remains trapped inside the cell (see Figure 12.4). This is exacerbated when sorbitol dehydrogenase is low or absent (for example, in retina, lens, kidney, and nerve cells). As a result, sorbitol accumulates in these cells, causing strong osmotic effects and, therefore, cell swelling as a result of water retention. Some of the pathologic alterations associated with diabetes can be attributed, in part, to this phenomenon, including cataract formation, peripheral neuropathy, and microvascular problems leading to nephropathy and retinopathy. (See p. 344 for a discussion of the complications of diabetes.) [Note: Use of NADPH in the aldose reductase reaction decreases the generation of reduced glutathione, an important antioxidant (see p. 148), and may be related to diabetic complications.]

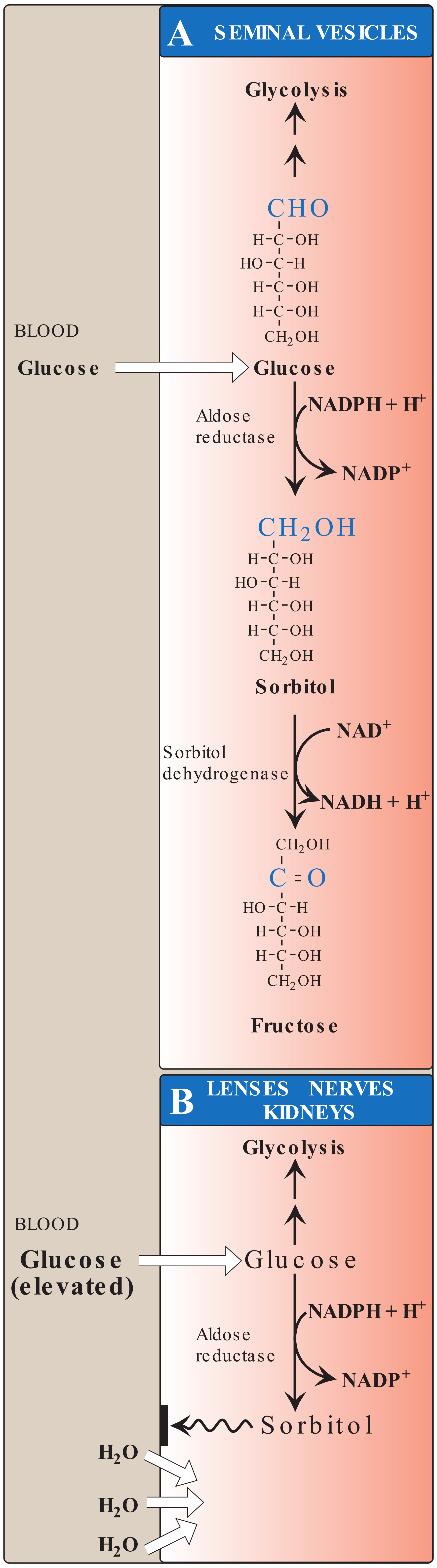

A SEMINAL VESICLES
Glycolysis
CHO
H-C-OH
HO-C-H
H-C-OH
H-C-OH
CH$_2$OH
BLOOD
Glucose
Glucose
Aldose reductase
NADPH + H$^+$
NADP$^+$
CH$_2$OH
H-C-OH
HO-C-H
H-C-OH
H-C-OH
CH$_2$OH
Sorbitol
Sorbitol dehydrogenase
NAD$^+$
NADH + H$^+$
CH$_2$OH
C = O
HO-C-H
H-C-OH
H-C-OH
CH$_2$OH
Fructose
B LENSES NERVES KIDNEYS
Glycolysis
BLOOD
Glucose (elevated)
Glucose
Aldose reductase
NADPH + H$^+$
NADP$^+$
Sorbitol
H$_2$O
H$_2$O
H$_2$O

Figure 12.4
Sorbitol metabolism. NAD(H) = nicotinamide adenine dinucleotide; NADP(H) = nicotinamide adenine dinucleotide phosphate.

III. GALACTOSE METABOLISM

The major dietary source of galactose is lactose (galactosyl β-1,4-glucose) obtained from milk and milk products. [Note: The digestion of lactose by *β*-galactosidase (lactase) of the intestinal mucosal cell membrane was discussed on p. 87.] Some galactose can also be obtained by lysosomal degradation of complex carbohydrates, such as glycoproteins and glycolipids, which are important membrane components. Like fructose, the transport of galactose into cells is not insulin dependent.

A. Phosphorylation of galactose

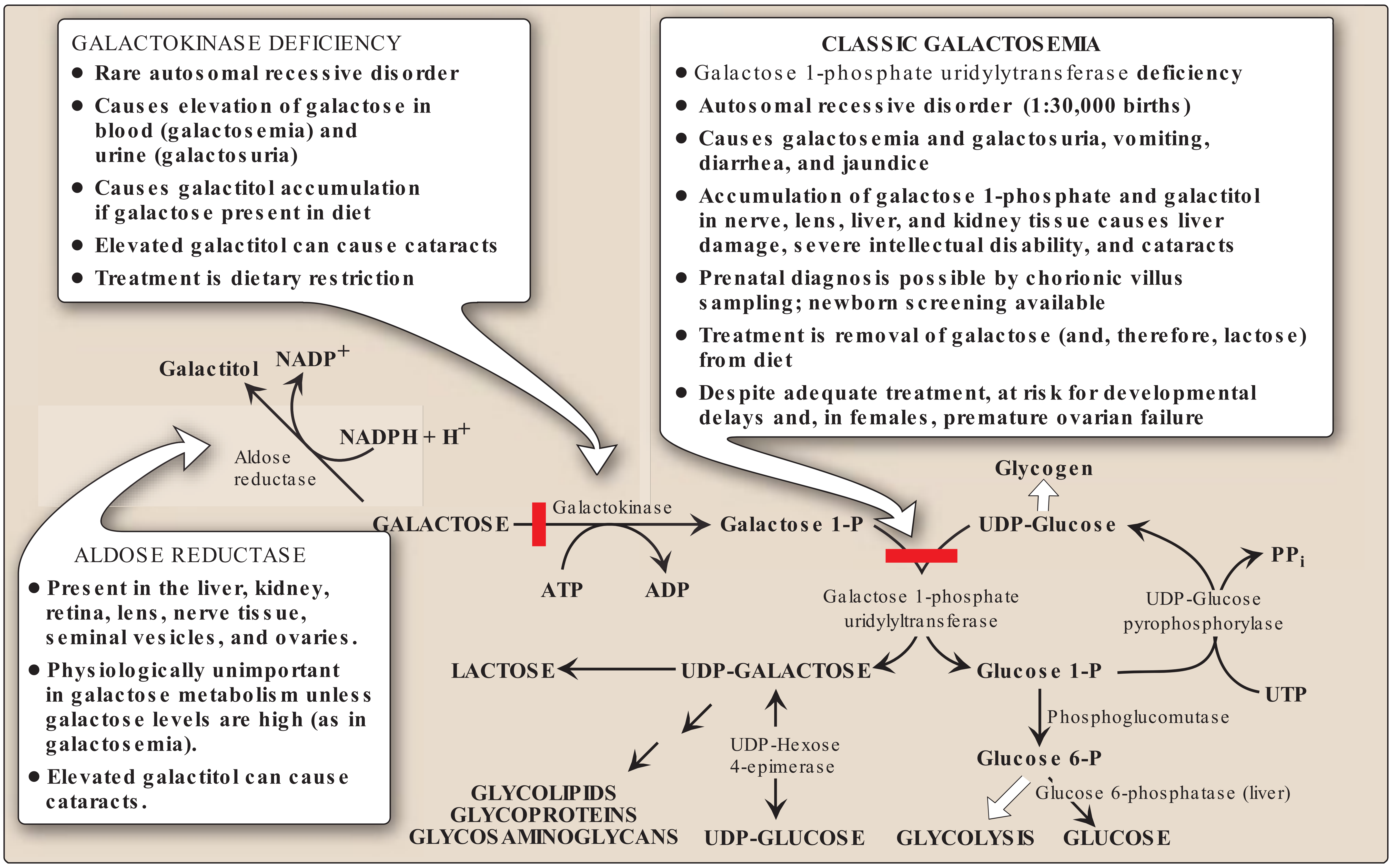
Like fructose, galactose must be phosphorylated before it can be further metabolized. Most tissues have a specific enzyme for this purpose, galactokinase, which produces galactose 1-phosphate (Figure 12.5). As with other kinases, ATP is the phosphate donor.

B. Formation of uridine diphosphate-galactose

Galactose 1-phosphate cannot enter the glycolytic pathway unless it is first converted to uridine diphosphate (UDP)-galactose (Figure 12.6). This occurs in an exchange reaction, in which UDP-glucose reacts with galactose 1-phosphate, producing UDP-galactose and glucose 1-phosphate (see Figure 12.5). The enzyme that catalyzes this reaction is galactose 1-phosphate uridylyltransferase (GALT).

C. Use of uridine diphosphate-galactose as a carbon source for glycolysis or gluconeogenesis

For UDP-galactose to enter the mainstream of glucose metabolism, it must first be converted to its C-4 epimer, UDP-glucose, by UDP-



GALACTOKINASE DEFICIENCY

- **Rare autosomal recessive disorder**
- **Causes elevation of galactose in blood (galactosemia) and urine (galactosuria)**
- **Causes galactitol accumulation if galactose present in diet**
- **Elevated galactitol can cause cataracts**
- **Treatment is dietary restriction**

CLASSIC GALACTOSEMIA

- Galactose 1-phosphate uridylyltransferase **deficiency**
- **Autosomal recessive disorder (1:30,000 births)**
- **Causes galactosemia and galactosuria, vomiting, diarrhea, and jaundice**
- **Accumulation of galactose 1-phosphate and galactitol in nerve, lens, liver, and kidney tissue causes liver damage, severe intellectual disability, and cataracts**
- **Prenatal diagnosis possible by chorionic villus sampling; newborn screening available**
- **Treatment is removal of galactose (and, therefore, lactose) from diet**
- **Despite adequate treatment, at risk for developmental delays and, in females, premature ovarian failure**

ALDOSE REDUCTASE

- **Present in the liver, kidney, retina, lens, nerve tissue, seminal vesicles, and ovaries.**
- **Physiologically unimportant in galactose metabolism unless galactose levels are high (as in galactosemia).**
- **Elevated galactitol can cause cataracts.**

Galactitol
NADP+
NADPH + H+
Aldose reductase
GALACTOSE
Galactokinase
ATP
ADP
Galactose 1-P
Glycogen
UDP-Glucose
PPi
Galactose 1-phosphate uridylyltransferase
UDP-Glucose pyrophosphorylase
LACTOSE
UDP-GALACTOSE
Glucose 1-P
UTP
Phosphoglucomutase
UDP-Hexose 4-epimerase
Glucose 6-P
Glucose 6-phosphatase (liver)
GLYCOLIPIDS
GLYCOPROTEINS
GLYCOSAMINOGLYCANS
UDP-GLUCOSE
GLYCOLYSIS
GLUCOSE

Figure 12.5
Metabolism of galactose. UDP = uridine diphosphate; UTP = uridine triphosphate; P = phosphate; PP_i = pyrophosphate; NADP(H) = nicotinamide adenine dinucleotide phosphate; ADP = adenosine diphosphate.

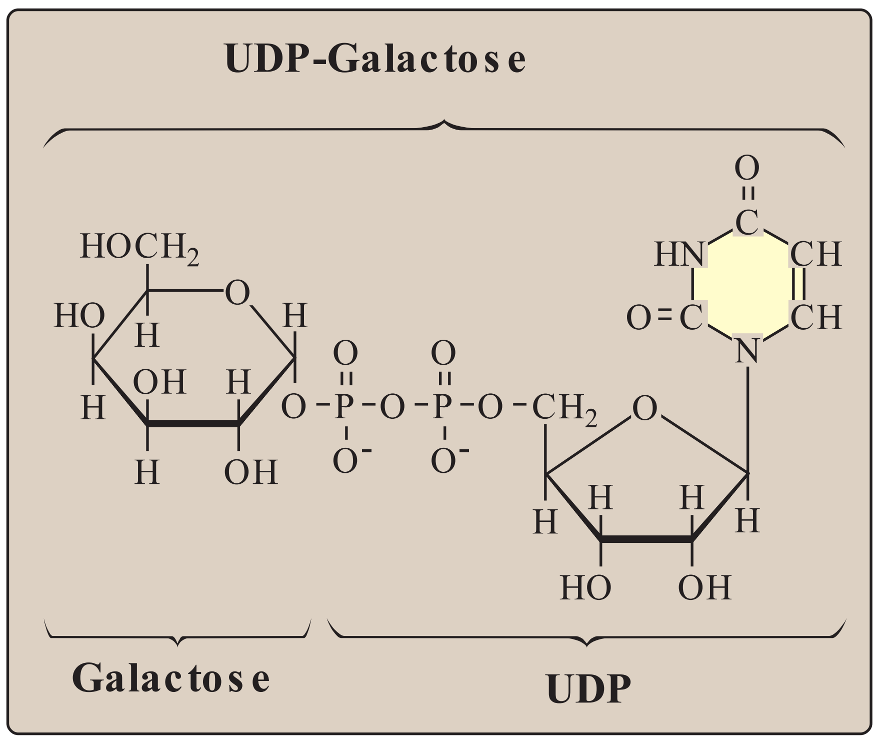
hexose 4-epimerase. This "new" UDP-glucose (produced from the original UDP-galactose) can then participate in many biosynthetic reactions as well as being used in the GALT reaction described above. (See Figure 12.5 for a summary of this interconversion.)

D. Role of uridine diphosphate-galactose in biosynthetic reactions

UDP-galactose can serve as the donor of galactose units in a number of synthetic pathways, including synthesis of lactose (see below), glycoproteins (see p. 166), glycolipids (see p. 210), and glycosaminoglycans (see p. 158). [Note: If galactose is not provided by the diet (for example, when it cannot be released from lactose as a result of a lack of β-galactosidase in people who are lactose intolerant), all tissue requirements for UDP-galactose can be met by the action of UDP-hexose 4-epimerase on UDP-glucose, which is efficiently produced from glucose 1-phosphate (see Figure 12.5).]

E. Disorders of galactose metabolism

GALT is deficient in individuals with classic galactosemia (see Figure 12.5). In this disorder, galactose 1-phosphate and, therefore, galactose accumulate. Physiologic consequences are similar to those found in hereditary fructose intolerance (see p. 138), but a broader spectrum of tissues is affected. The accumulated galactose is shunted into side pathways such as that of galactitol production.



UDP-Galactose
Galactose
UDP

Figure 12.6
Structure of UDP-galactose. UDP = uridine diphosphate.

This reaction is catalyzed by aldose reductase, the same enzyme that converts glucose to sorbitol (see p. 139). Treatment requires removal of galactose and lactose from the diet. GALT deficiency is part of the newborn screening panel. [Note: A deficiency in galactokinase results in a less severe disorder of galactosemia metabolism, although cataracts are common (see Figure 12.5).]

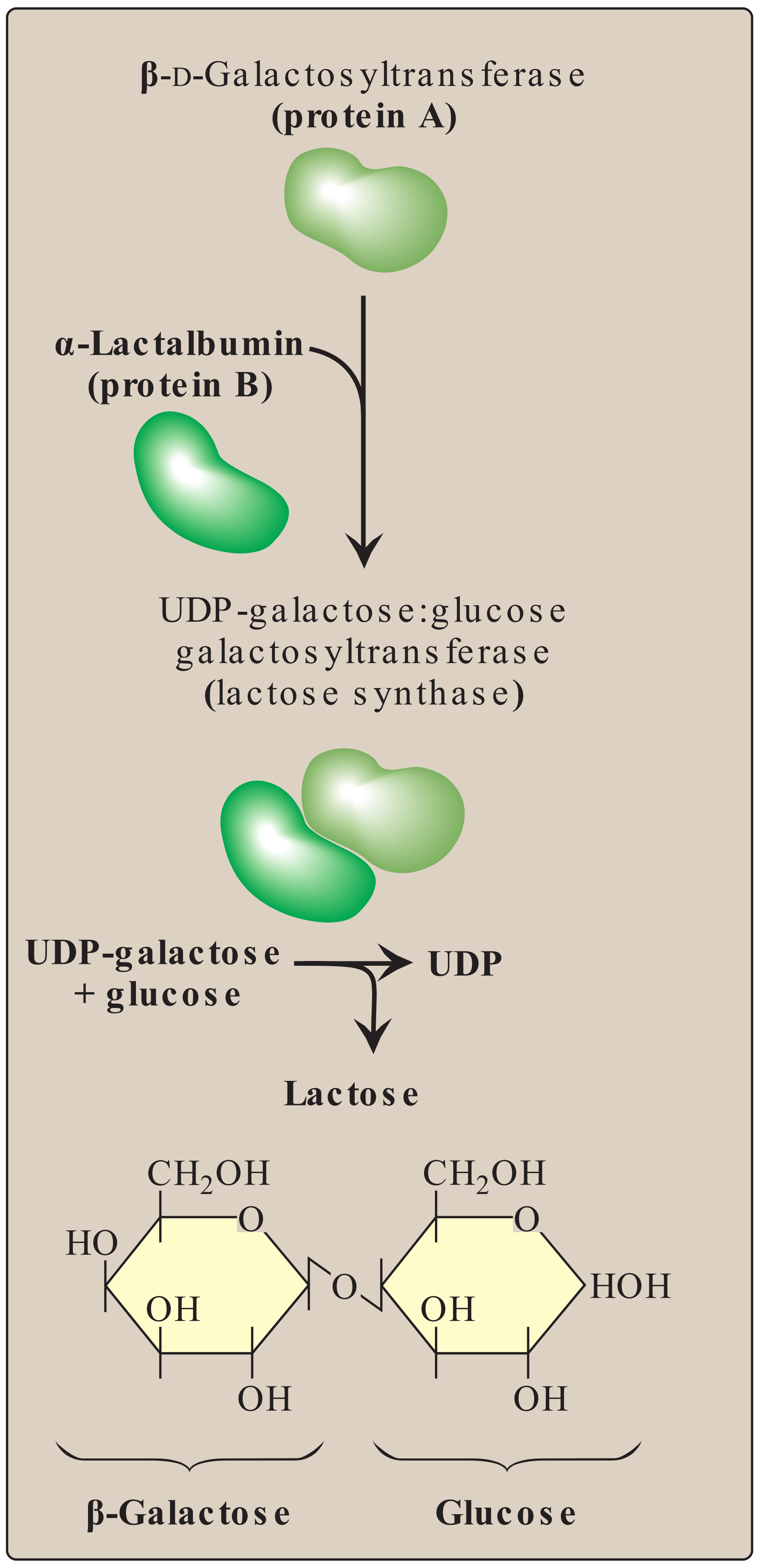


Figure 12.7
Lactose synthesis. UDP = uridine diphosphate.

IV. LACTOSE SYNTHESIS

Lactose is a disaccharide that consists of a molecule of β-galactose attached by a β(1→4) linkage to glucose. Therefore, lactose is galactosyl β(1→4)-glucose. Lactose, known as "milk sugar," is made by lactating (milk-producing) mammary glands. Therefore, milk and other dairy products are the dietary sources of lactose. Lactose is synthesized in the Golgi by lactose synthase (UDP-galactose:glucose galactosyltransferase), which transfers galactose from UDP-galactose to glucose, releasing UDP (Figure 12.7). This enzyme is composed of two proteins, A and B. Protein A is a *β*-D-galactosyltransferase and is found in a number of body tissues. In tissues other than the lactating mammary gland, this enzyme transfers galactose from UDP-galactose to N-acetyl-D-glucosamine, forming the same β(1→4) linkage found in lactose, and producing N-acetyllactosamine, a component of the structurally important N-linked glycoproteins (see p. 167). In contrast, protein B is found only in lactating mammary glands. It is α-lactalbumin, and its synthesis is stimulated by the peptide hormone prolactin. Protein B forms a complex with the enzyme, protein A, changing the specificity of that transferase so that lactose, rather than N-acetyllactosamine, is produced (see Figure 12.7).

V. CHAPTER SUMMARY

The major source of fructose is **sucrose**, which, when cleaved, releases equimolar amounts of fructose and glucose (Figure 12.8). Transport of fructose into cells is **insulin independent**. Fructose is first phosphorylated to **fructose 1-phosphate** by fructokinase and then cleaved by aldolase B to **dihydroxyacetone phosphate** and **glyceraldehyde**. These enzymes are found in the **liver**, **kidney**, and **small intestinal mucosa**. A **deficiency** of fructokinase causes a benign condition (**essential fructosuria**), but a **deficiency** of aldolase B causes **hereditary fructose intolerance (HFI)**, in which **severe hypoglycemia** and **liver failure** lead to **death** if fructose (and sucrose) in the diet is not eliminated. **Mannose**, an important component of **glycoproteins**, is phosphorylated by hexokinase to **mannose 6-phosphate**, which is reversibly isomerized to **fructose 6-phosphate** by phosphomannose isomerase. **Glucose** can be reduced to **sorbitol** (**glucitol**) by aldose reductase in many tissues, including the **lens**, **retina**, **Schwann cells**, **liver**, **kidney**, **ovaries**, and **seminal vesicles**. In cells of the **liver**, **ovaries**, and **seminal vesicles**, a second enzyme, sorbitol dehydrogenase, can oxidize sorbitol to produce **fructose**. **Hyperglycemia** results in the accumulation of sorbitol in those cells lacking sorbitol dehydrogenase. The resulting **osmotic events** cause cell swelling and may contribute to the **cataract formation**, **peripheral neuropathy**, **nephropathy**, and **retinopathy** seen in **diabetes**. The major dietary source of galactose is **lactose**. The transport of galactose into cells is not insulin dependent. Galactose is first phosphorylated by galactokinase (a deficiency results in cataracts) to **galactose 1-phosphate**. This compound is converted to **uridine diphosphate (UDP)-galactose** by galactose 1-phosphate uridyltransferase (GALT), with the nucleotide supplied by UDP-glucose. A **deficiency** of this enzyme causes **classic galactosemia**. Galactose 1-phosphate accumulates, and excess galactose is converted to **galactitol** by aldose reductase. This causes **liver damage**, **severe intellectual disability**, and **cataracts**. Treatment requires removal of galactose (and lactose) from the diet. For UDP-galactose to enter the mainstream of glucose metabolism, it must first be converted to UDP-glucose by UDP-hexose 4-epimerase. This enzyme can also be used to produce UDP-galactose from UDP-glucose when the former is required for the synthesis of structural carbohydrates. **Lactose** is a disaccharide that consists of **galactose** and **glucose**. Milk and other dairy products are the dietary sources of lactose. Lactose is synthesized by lactose synthase from **UDP-galactose** and **glucose** in the **lactating mammary gland**. The enzyme has two subunits, **protein A** (which is a galactosyltransferase found in most cells where it synthesizes **N-acetyllactosamine**) and **protein B** (**α-lactalbumin**, which is found only in the lactating mammary glands, and whose synthesis is stimulated by the peptide hormone **prolactin**). When both subunits are present, the transferase produces lactose.

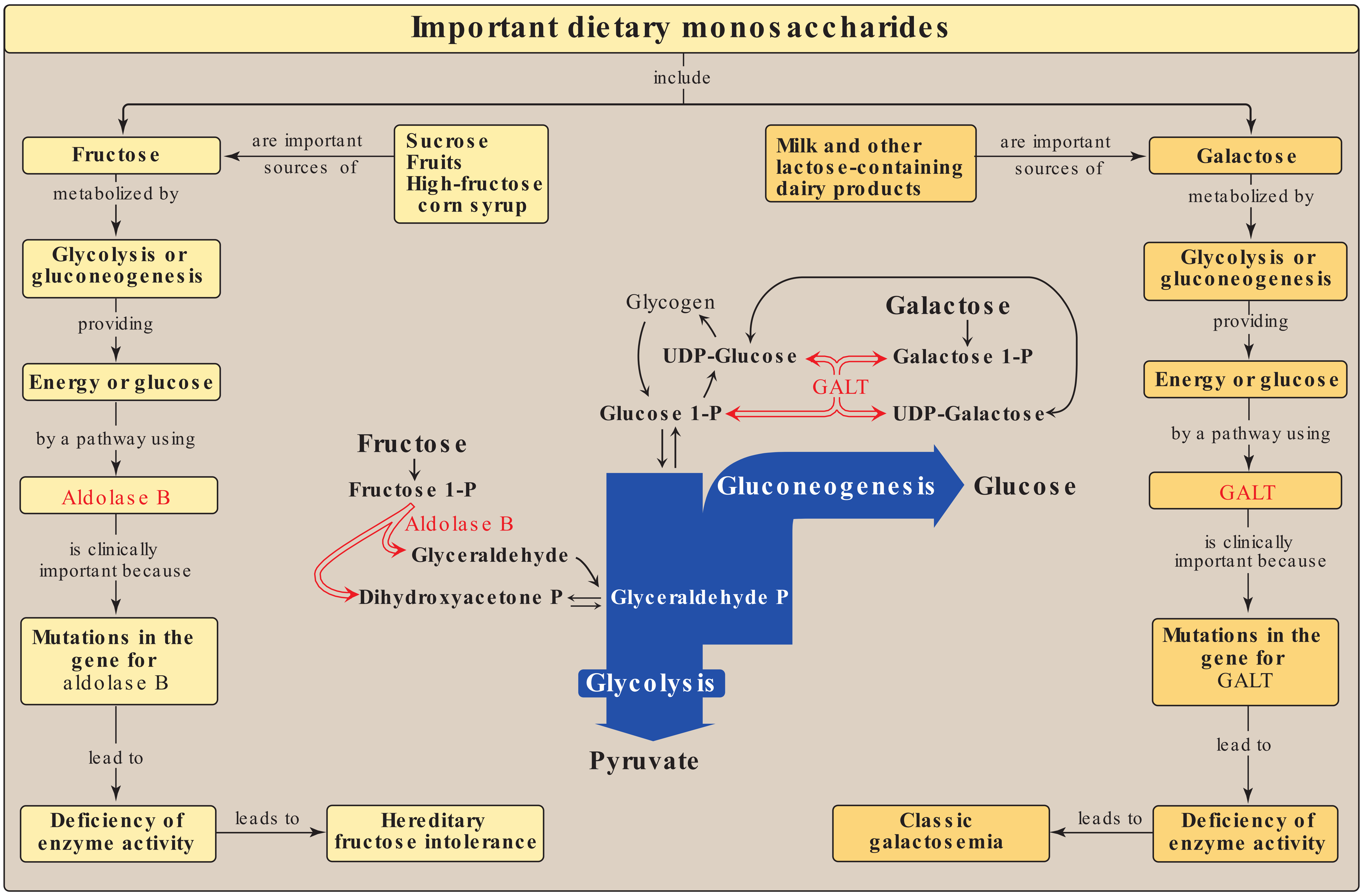

Important dietary monosaccharides
include
Fructose
are important sources of
Sucrose
Fruits
High-fructose corn syrup
Milk and other lactose-containing dairy products
are important sources of
Galactose
metabolized by
Glycolysis or gluconeogenesis
providing
Energy or glucose
by a pathway using
Aldolase B
is clinically important because
Mutations in the gene for aldolase B
lead to
Deficiency of enzyme activity
leads to
Hereditary fructose intolerance
metabolized by
Glycolysis or gluconeogenesis
providing
Energy or glucose
by a pathway using
GALT
is clinically important because
Mutations in the gene for GALT
lead to
Deficiency of enzyme activity
leads to
Classic galactosemia
Glycogen
Galactose
UDP-Glucose
Galactose 1-P
GALT
Glucose 1-P
UDP-Galactose
Fructose
Fructose 1-P
Aldolase B
Glyceraldehyde
Dihydroxyacetone P
Glyceraldehyde P
Gluconeogenesis
Glucose
Glycolysis
Pyruvate

Figure 12.8
Key concept map for metabolism of fructose and galactose. GALT = galactose 1-phosphate uridylyltransferase; UDP = uridine diphosphate; P = phosphate.

Pentose Phosphate Pathway and Nicotinamide Adenine Dinucleotide Phosphate

13

I. OVERVIEW

The pentose phosphate pathway (also called the hexose monophosphate shunt) occurs in the cytosol of the cell. It includes two irreversible oxidative reactions, followed by a series of reversible sugar–phosphate interconversions (Figure 13.1). No adenosine triphosphate (ATP) is directly consumed or produced in the cycle. Carbon 1 of glucose 6-phosphate is released as CO_2, and two reduced nicotinamide adenine dinucleotide phosphates (NADPHs) are produced for each glucose 6-phosphate molecule entering the oxidative part of the pathway. The rate and direction of the reversible reactions of the pentose phosphate pathway are determined by the supply of and demand for intermediates of the cycle. The pathway provides a major portion of the body's NADPH, which functions as a biochemical reductant. It also produces ribose 5-phosphate, required for the biosynthesis of nucleotides (see p. 293), and provides a mechanism for the metabolic use of five-carbon sugars obtained from the diet or the degradation of structural carbohydrates.

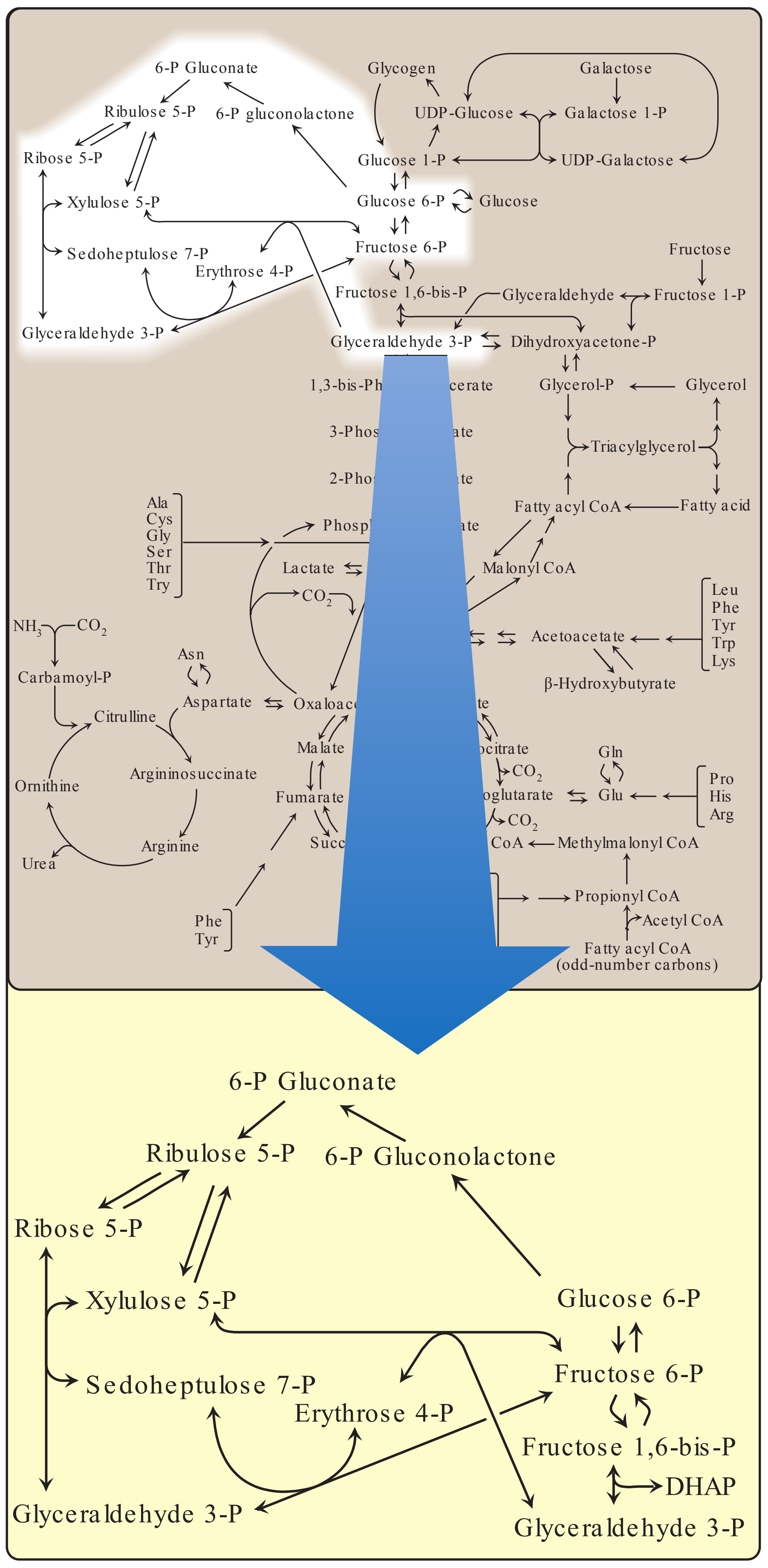

6-P Gluconate
Ribulose 5-P
6-P Gluconolactone
Ribose 5-P
Xylulose 5-P
Glucose 6-P
Sedoheptulose 7-P
Fructose 6-P
Erythrose 4-P
Fructose 1,6-bis-P
DHAP
Glyceraldehyde 3-P
Glyceraldehyde 3-P
Glycogen
UDP-Glucose
Galactose
Galactose 1-P
Glucose 1-P
UDP-Galactose
Glucose
Fructose
Glyceraldehyde
Fructose 1-P
Dihydroxyacetone-P
Glycerol-P
Glycerol
Triacylglycerol
Fatty acyl CoA
Fatty acid
Malonyl CoA
Acetoacetate
β-Hydroxybutyrate
Leu
Phe
Tyr
Trp
Lys
Ala
Cys
Gly
Ser
Thr
Try
Lactate
NH3
CO2
Carbamoyl-P
Asn
Aspartate
Citrulline
Argininosuccinate
Ornithine
Arginine
Urea
Malate
Fumarate
Gln
Glu
Pro
His
Arg
Methylmalonyl CoA
Propionyl CoA
Acetyl CoA
Fatty acyl CoA (odd-number carbons)
Phe
Tyr

Figure 13.1
Pentose phosphate pathway shown as a component of the metabolic map (see Figure 8.2, p. 92 for a more detailed view of the metabolic pathways). P = phosphate; DHAP = dihydroxyacetone phosphate.

II. IRREVERSIBLE OXIDATIVE REACTIONS

The oxidative portion of the pentose phosphate pathway consists of three reactions that lead to the formation of ribulose 5-phosphate, CO_2, and two molecules of NADPH for each molecule of glucose 6-phosphate oxidized (Figure 13.2). This portion of the pathway is particularly important in the liver, lactating mammary glands, and adipose tissue, which are active in the NADPH-dependent biosynthesis of fatty acids (see p. 186); in the testes, ovaries, placenta, and adrenal cortex, which are active in the NADPH-dependent biosynthesis of steroid hormones (see p. 237); and in red blood cells (RBCs), which require NADPH to keep glutathione reduced (see p. 152).

A. Dehydrogenation of glucose 6-phosphate

Glucose 6-phosphate dehydrogenase (G6PD) catalyzes an irreversible oxidation of glucose 6-phosphate to 6-phosphogluconolactone in a reaction that is specific for oxidized NADP ($NADP^+$) as the coenzyme. The pentose phosphate pathway is regulated primarily at the G6PD reaction. NADPH is a potent competitive inhibitor of the enzyme, and, under most metabolic conditions, the ratio of NADPH/

$NADP^+$ is sufficiently high to substantially inhibit enzyme activity. However, with increased demand for NADPH, the ratio of NADPH/$NADP^+$ decreases, and flux through the cycle increases in response to the enhanced activity of G6PD. Insulin upregulates expression of the gene for G6PD, and flux through the pathway increases in the absorptive state (see p. 323).

B. Formation of ribulose 5-phosphate

6-Phosphogluconolactone is hydrolyzed by 6-phosphogluconolactone hydrolase. The reaction is irreversible and not rate limiting. The oxidative decarboxylation of the product, 6-phosphogluconate, is catalyzed by 6-phosphogluconate dehydrogenase. This irreversible reaction produces a pentose sugar–phosphate (ribulose 5-phosphate), CO_2 (from carbon 1 of glucose), and a second molecule of NADPH (see Figure 13.2).

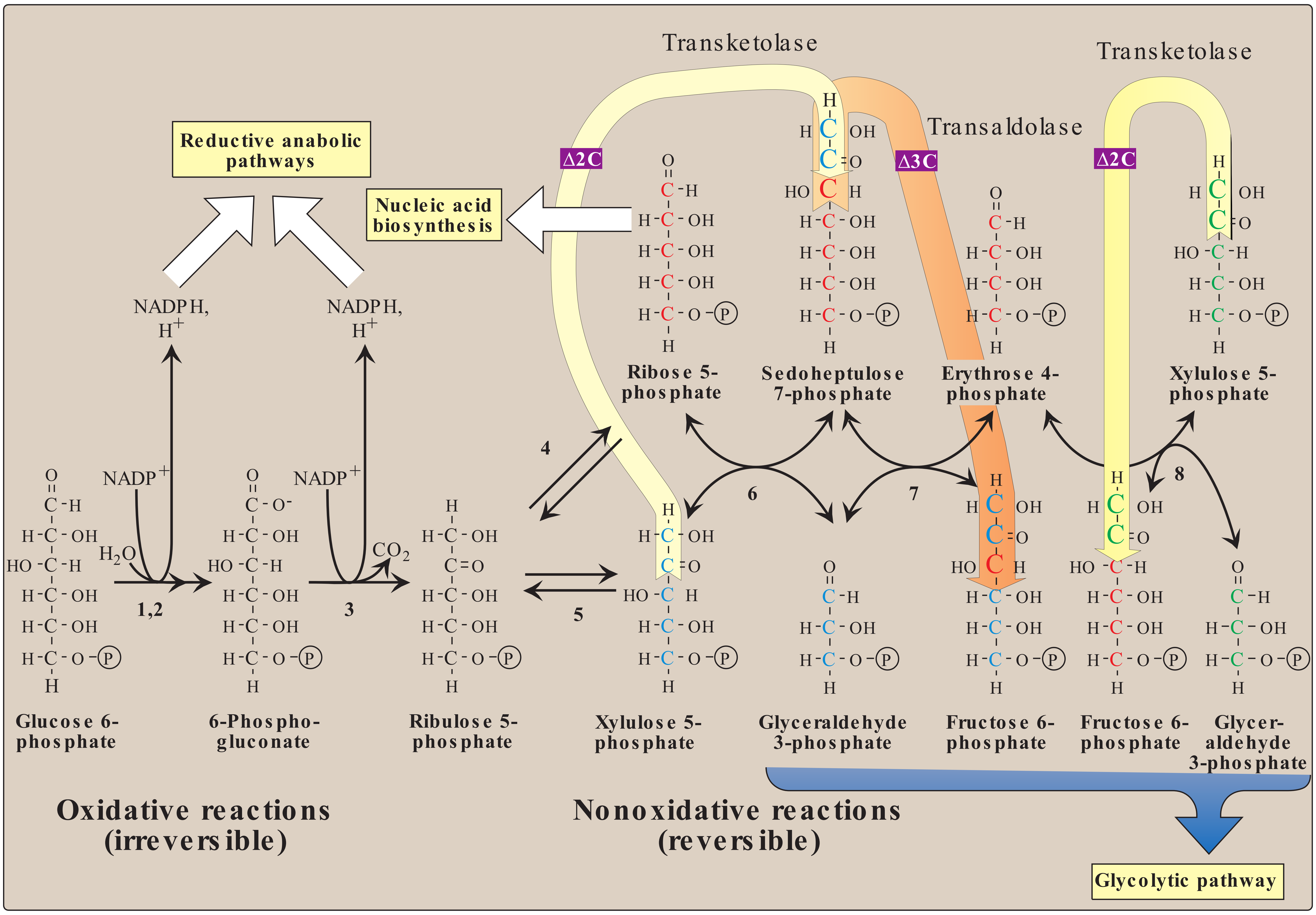

Transketolase
Transketolase
Transaldolase
Δ2C
Δ3C
Δ2C
Reductive anabolic pathways
Nucleic acid biosynthesis
NADPH, H+
NADPH, H+
NADP+
NADP+
H2O
CO2
1,2
3
4
5
6
7
8
Ribose 5-phosphate
Sedoheptulose 7-phosphate
Erythrose 4-phosphate
Xylulose 5-phosphate
Glucose 6-phosphate
6-Phosphogluconate
Ribulose 5-phosphate
Xylulose 5-phosphate
Glyceraldehyde 3-phosphate
Fructose 6-phosphate
Fructose 6-phosphate
Glyceraldehyde 3-phosphate
Oxidative reactions (irreversible)
Nonoxidative reactions (reversible)
Glycolytic pathway

Figure 13.2
Reactions of the pentose phosphate pathway. Enzymes numbered above are: 1, 2) glucose 6-phosphate dehydrogenase and 6-phosphogluconolactone hydrolase, 3) 6-phosphogluconate dehydrogenase, 4) ribose 5-phosphate isomerase, 5) phosphopentose epimerase, 6 and 8) transketolase (coenzyme: thiamine pyrophosphate), and 7) transaldolase. **Δ2C** = two carbons are transferred in transketolase reactions; **Δ3C** = three carbons are transferred in the transaldolase reaction. This can be represented as: 5C sugar + 5C sugar $\xrightarrow{\Delta 2C}$ 7C sugar + 3C sugar $\xrightarrow{\Delta 3C}$ 4C sugar + 6C sugar. NADP(H) = nicotinamide adenine dinucleotide phosphate; P = phosphate.

III. REVERSIBLE NONOXIDATIVE REACTIONS

The nonoxidative reactions of the pentose phosphate pathway occur in all cell types synthesizing nucleotides and nucleic acids. These reactions catalyze the interconversion of sugars containing three to seven carbons (see Figure 13.2). These reversible reactions permit ribulose 5-phosphate (produced by the oxidative portion of the pathway) to be converted either to ribose 5-phosphate (needed for nucleotide synthesis; see p. 293) or to intermediates of glycolysis (that is, fructose 6-phosphate and glyceraldehyde 3-phosphate). For example, many cells that carry out reductive biosynthetic reactions have a greater need for NADPH than for ribose 5-phosphate. In this case, transketolase (which transfers two-carbon units in a thiamine pyrophosphate [TPP]-requiring reaction)and transaldolase (which transfers three-carbon units) convert the ribulose 5-phosphate produced as an end product of the oxidative reactions to glyceraldehyde 3-phosphate and fructose 6-phosphate, which are glycolytic intermediates. In contrast, when the demand for ribose for nucleotides and nucleic acids is greater than the need for NADPH, the nonoxidative reactions can provide the ribose 5-phosphate from glyceraldehyde 3-phosphate and fructose 6-phosphate in the absence of the oxidative steps (Figure 13.3).

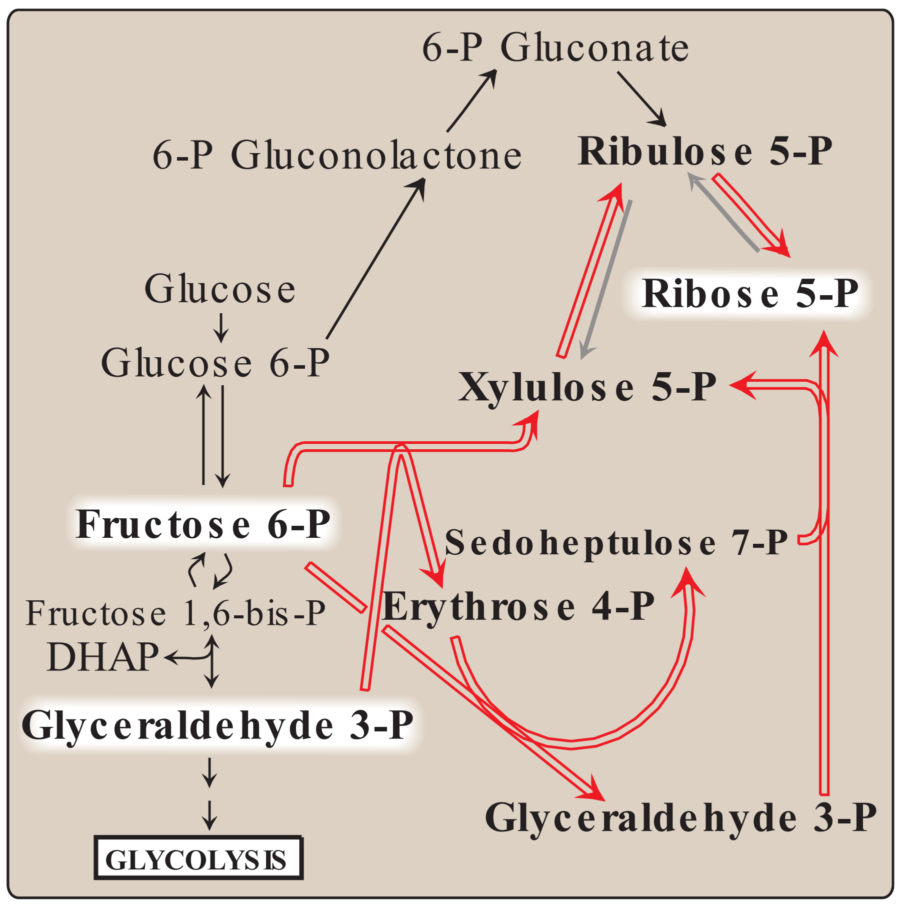


Figure 13.3
Formation of ribose 5-phosphate from intermediates of glycolysis. P = phosphate; DHAP = dihydroxyacetone phosphate.

> In addition to transketolases, TPP is required by the enzyme complexes pyruvate dehydrogenase (see p. 110), α-ketoglutarate dehydrogenase of the citric acid cycle (see p. 112), and branched-chain α-keto acid dehydrogenase of branched-chain amino acid catabolism (see p. 266).

IV. USES OF NADPH

The coenzyme NADPH differs from nicotinamide adenine dinucleotide (NADH) only by the presence of a phosphate group on one of the ribose units (Figure 13.4). This seemingly small change in structure allows NADPH to interact with NADPH-specific enzymes that have unique roles in the cell. For example, in the cytosol of hepatocytes the steady-state ratio of NADP$^+$/NADPH is approximately 0.1, which favors the use of NADPH in reductive biosynthetic reactions. This contrasts with the high ratio of NAD$^+$/NADH (approximately 1000), which favors an oxidative role for NAD$^+$. This section summarizes some important NADP$^+$ and NADPH-specific functions in reductive biosynthesis and detoxification reactions.

A. Reductive biosynthesis

NADPH can be thought of as a high-energy molecule, much in the same way as NADH. However, the electrons of NADPH are destined for use in reductive biosynthesis, rather than for transfer to oxygen as is the case with NADH (see p. 74). Thus, in the metabolic transformations of the pentose phosphate pathway, part of the energy of glucose 6-phosphate is conserved in NADPH, a molecule with a negative reduction potential (see p. 77), that, therefore, can be used in reactions requiring an electron donor, such as fatty acid (see p. 186) and steroid (see p. 237) synthesis.

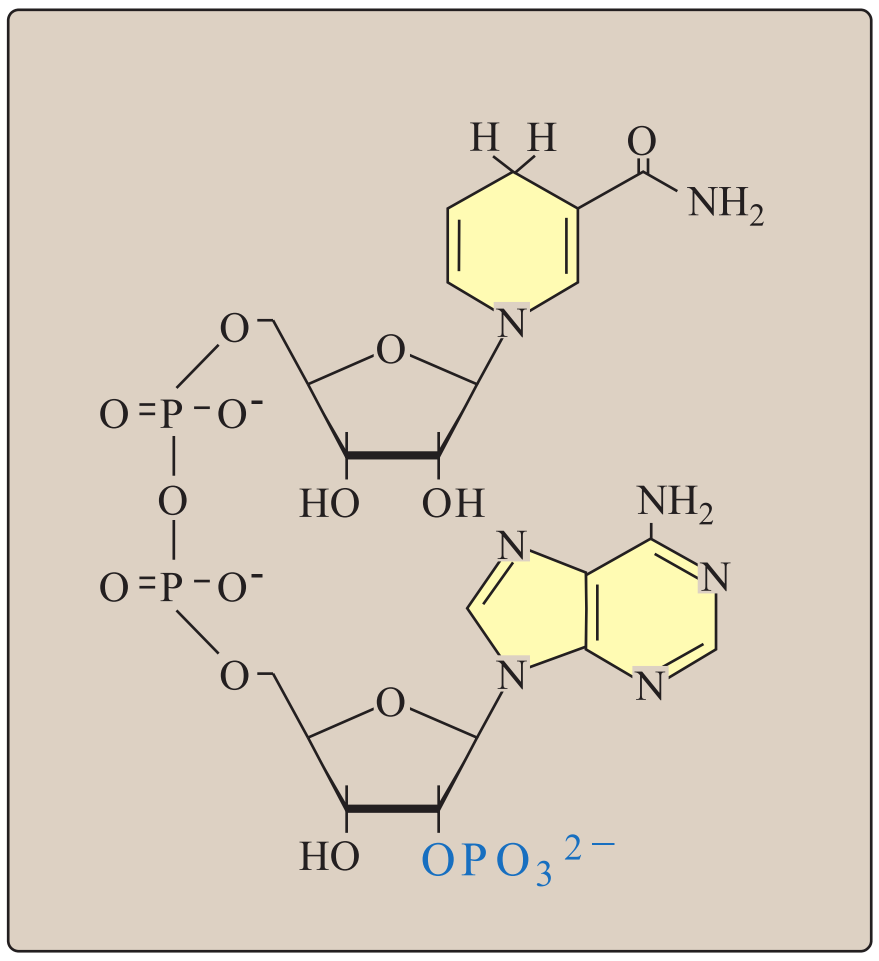


Figure 13.4
Structure of reduced nicotinamide adenine dinucleotide phosphate (NADPH).

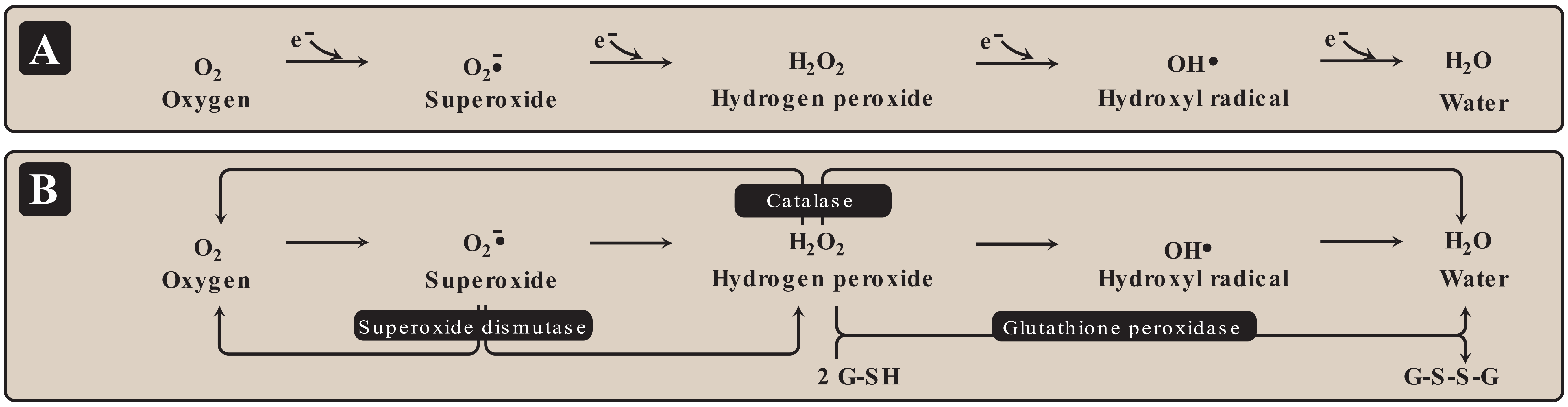

A
O_2
Oxygen
e⁻
$O_2^{\bar{\bullet}}$
Superoxide
e⁻
H_2O_2
Hydrogen peroxide
e⁻
OH•
Hydroxyl radical
e⁻
H_2O
Water
B
Catalase
O_2
Oxygen
$O_2^{\bar{\bullet}}$
Superoxide
H_2O_2
Hydrogen peroxide
OH•
Hydroxyl radical
H_2O
Water
Superoxide dismutase
Glutathione peroxidase
2 G-SH
G-S-S-G

Figure 13.5

A. Formation of reactive intermediates from molecular oxygen. e⁻ = electrons. B. Actions of antioxidant enzymes. G-SH = reduced glutathione; G-S-S-G = oxidized glutathione. (See Figure 13.6B for the regeneration of G-SH.)

B. Reduction of hydrogen peroxide

Hydrogen peroxide (H_2O_2) is one of a family of reactive oxygen species (ROS) that are formed from the partial reduction of molecular oxygen (Figure 13.5A). These compounds are formed continuously as byproducts of aerobic metabolism, through reactions with drugs and environmental toxins, or when the level of antioxidants is diminished, all creating the condition of oxidative stress. The highly reactive oxygen intermediates can cause serious chemical damage to DNA, proteins, and unsaturated lipids and can lead to cell death. ROS have been implicated in a number of pathologic processes, including reperfusion injury, cancer, inflammatory disease, and aging. The cell has several protective mechanisms that minimize the toxic potential of these compounds.

1. **Enzymes that catalyze antioxidant reactions:** Reduced glutathione (G-SH), a tripeptide-thiol (γ-glutamylcysteinylglycine) present in most cells, can chemically detoxify H_2O_2 (Figure 13.5B). This reaction, catalyzed by the selenium-containing glutathione peroxidase, forms oxidized glutathione (G-S-S-G), which no longer has protective properties. The cell regenerates G-SH in a reaction catalyzed by glutathione reductase, using NADPH as a source of reducing equivalents. Thus, NADPH indirectly provides electrons for the reduction of H_2O_2 (Figure 13.6). [Note: RBCs are totally dependent on the pentose phosphate pathway for their supply of NADPH because, unlike other cell types, RBCs do not have an alternate source for this essential coenzyme.] Additional enzymes, such as superoxide dismutase and catalase, catalyze the conversion of other reactive oxygen intermediates to harmless products (see Figure 13.5B). As a group, these enzymes serve as a defense system to guard against the toxic effects of ROS.

2. **Antioxidant chemicals:** A number of intracellular reducing agents, such as ascorbate (see p. 377), vitamin E (see p. 391), and β-carotene (see p. 382), are able to reduce and, thereby, detoxify reactive oxygen intermediates in the laboratory. Consumption of foods rich in these antioxidant compounds has been correlated with a reduced risk for certain types of cancers as well as decreased frequency of certain other chronic health

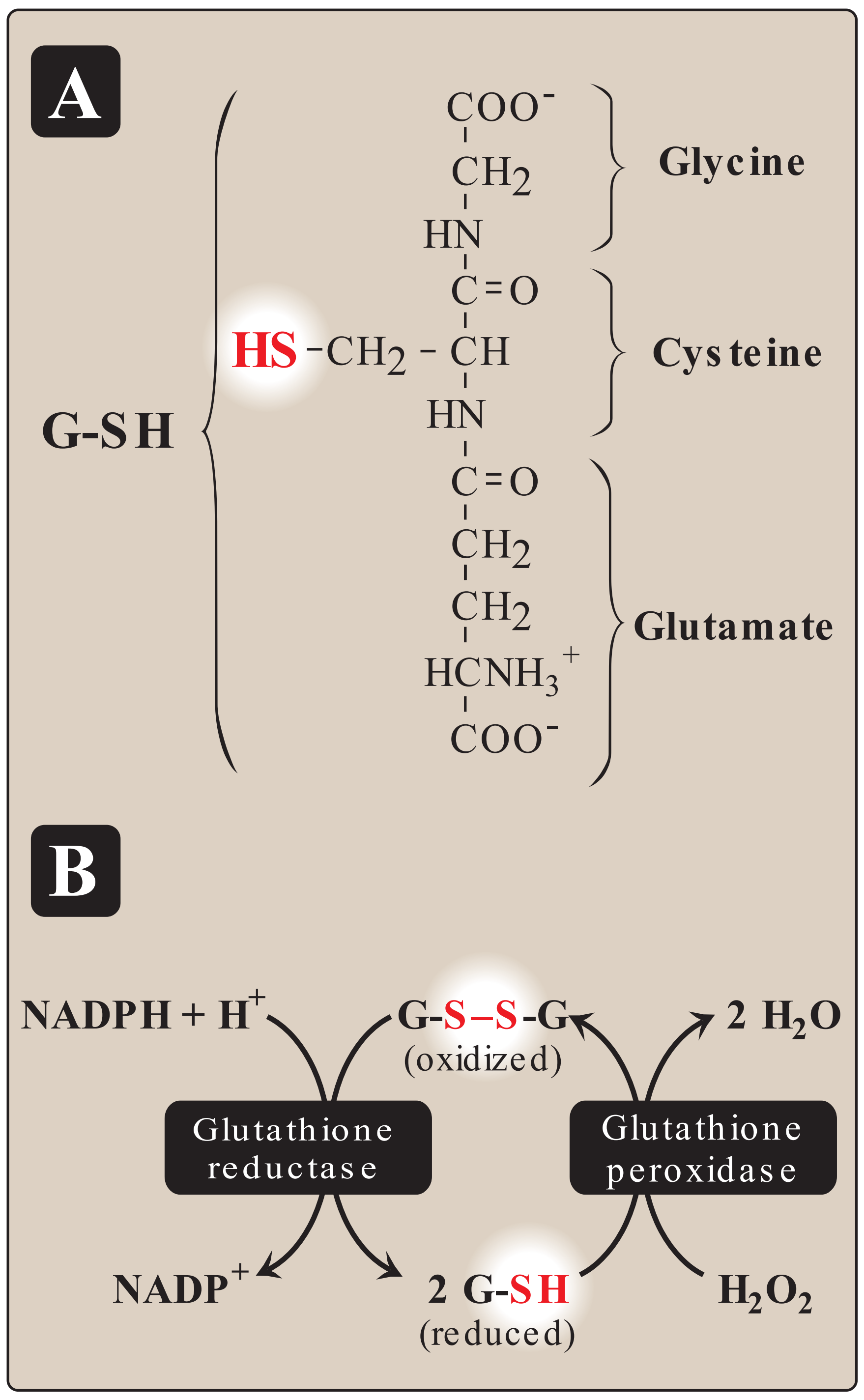

A
G-SH
COO^-
CH_2
HN
C=O
HS
$-CH_2-CH$
HN
C=O
CH_2
CH_2
$HCNH_3^+$
COO^-
Glycine
Cysteine
Glutamate
B
NADPH + H^+
G-S-S-G
(oxidized)
2 H_2O
Glutathione reductase
Glutathione peroxidase
$NADP^+$
2 G-SH
(reduced)
H_2O_2

Figure 13.6

A. Structure of reduced glutathione (G-SH). [Note: Glutamate is linked to cysteine through a γ-carboxyl, rather than an α-carboxyl.]
B. Glutathione-mediated reduction of hydrogen peroxide (H_2O_2) by reduced nicotinamide adenine dinucleotide phosphate (NADPH). G-S-S-G = oxidized glutathione.

problems. Therefore, it is tempting to speculate that the effects of these compounds are, in part, an expression of their ability to quench the toxic effect of ROS. However, clinical trials with antioxidants as dietary supplements have failed to show clear beneficial effects. In the case of dietary supplementation with β-carotene, the rate of lung cancer in smokers increased rather than decreased. Thus, the health-promoting effects of dietary fruits and vegetables likely reflect a complex interaction among many naturally occurring compounds, which has not been duplicated by consumption of isolated antioxidant compounds.

C. Cytochrome P450 monooxygenase system

Monooxygenases (mixed-function oxidases) incorporate one atom from molecular oxygen into a substrate (creating a hydroxyl group), with the other atom being reduced to water. In the cytochrome P450 monooxygenase system, NADPH provides the reducing equivalents required by this series of reactions (Figure 13.7). This system performs different functions in two separate locations in cells. The overall reaction catalyzed by a cytochrome P450 enzyme is:

$$\text{R-H} + O_2 + \text{NADPH} + H^+ \rightarrow \text{R-OH} + H_2O + \text{NADP}^+$$

where R may be a steroid, drug, or other chemical. [Note: Cytochrome P450 (CYP) enzymes are actually a superfamily of related, heme-containing monooxygenases that participate in a broad variety of reactions. The P450 in the name reflects the absorbance at 450 nm by the protein.]

1. **Mitochondrial system:** An important function of the cytochrome P450 monooxygenase system found associated with the inner mitochondrial membrane is the biosynthesis of steroid hormones. In steroidogenic tissues, such as the placenta, ovaries, testes, and adrenal cortex, it is used to hydroxylate intermediates in the conversion of cholesterol to steroid hormones, a process that makes these hydrophobic compounds more water soluble (see p. 237). The liver uses this same system in bile acid synthesis (see p. 224) and the hydroxylation of cholecalciferol to 25-hydroxycholecalciferol (vitamin D_3; see p. 386), and the kidney uses it to hydroxylate vitamin D_3 to its biologically active 1,25-dihydroxylated form.

2. **Microsomal system:** An extremely important function of the microsomal cytochrome P450 monooxygenase system found associated with the membrane of the smooth endoplasmic reticulum (particularly in the liver) is the detoxification of foreign compounds (xenobiotics). These include numerous drugs and such varied pollutants as petroleum products and pesticides. CYP enzymes of the microsomal system (for example, CYP3A4), can be used to hydroxylate these toxins. The purpose of these modifications is two-fold. First, it may itself activate or inactivate a drug and second, make a toxic compound more soluble, thereby facilitating its excretion in the urine or feces. Frequently, however, the new hydroxyl group will serve as a site for conjugation with a polar molecule, such as glucuronic acid (see p. 161), which will significantly increase the compound's solubility. [Note: Polymorphisms (see p.473) in the genes for CYP enzymes can lead to differences in drug metabolism.]

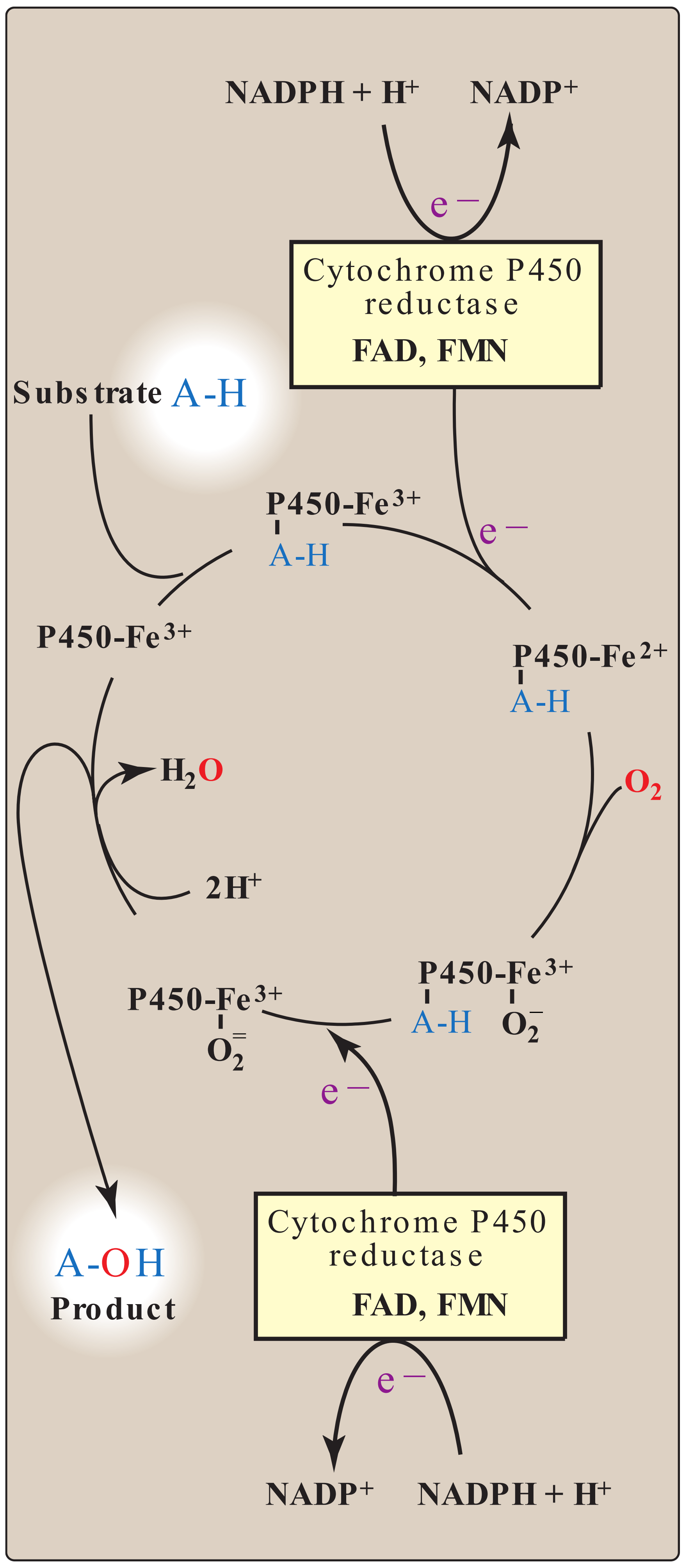


Figure 13.7
Cytochrome P450 monooxygenase cycle (simplified). Electrons (e^-) move from NADPH to FAD to FMN of the reductase and then to the heme iron (Fe) of the P450 enzyme. [Note: In the mitochondrial system, electrons move from FAD to an iron-sulfur protein and then to the P450 enzyme.] FAD = flavin adenine dinucleotide; FMN = flavin mononucleotide; NADPH = reduced nicotinamide adenine dinucleotide phosphate.

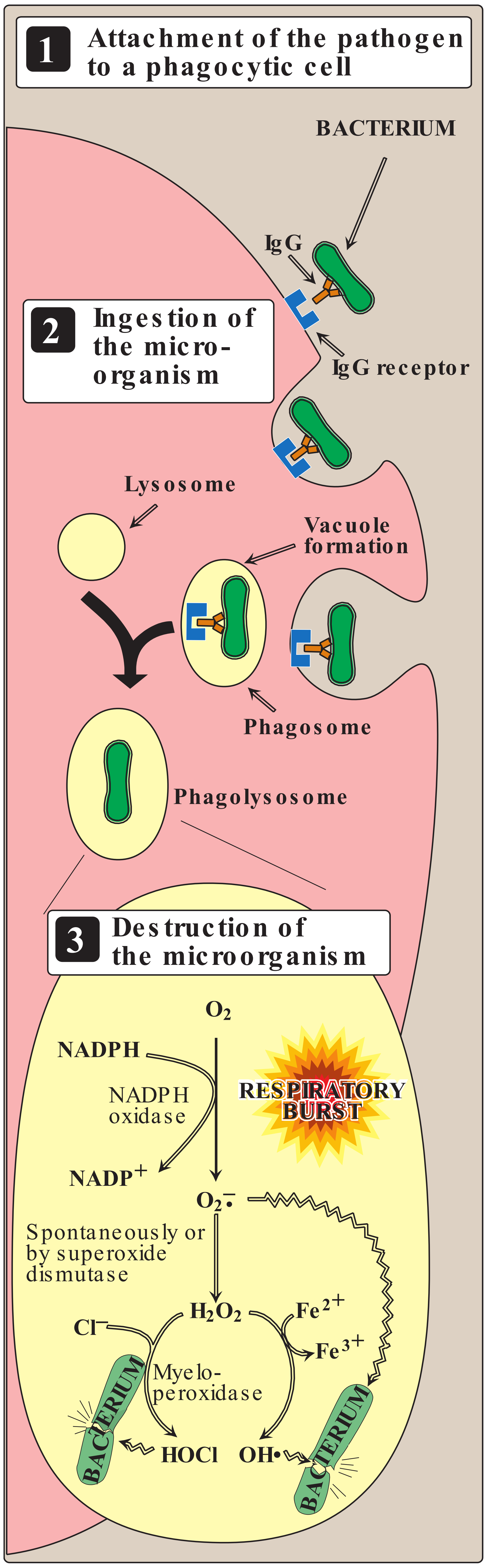


Figure 13.8
Phagocytosis and the oxygen-dependent pathway of microbial killing. IgG = the antibody immunoglobulin G; NADPH = reduced nicotinamide adenine dinucleotide phosphate; $O_2^{\bar{\bullet}}$ = superoxide; HOCl = hypochlorous acid; OH• = hydroxyl radical.

D. Phagocytosis by white blood cells

Phagocytosis is the ingestion by receptor-mediated endocytosis of microorganisms, foreign particles, and cellular debris by cells such as neutrophils and macrophages (monocytes). It is an important defense mechanism, particularly in bacterial infections. Neutrophils and monocytes are armed with both oxygen-independent and oxygen-dependent mechanisms for killing bacteria.

1. **Oxygen-independent mechanism:** Oxygen-independent mechanisms use pH changes in phagolysosomes and lysosomal enzymes to destroy pathogens.

2. **Oxygen-dependent system:** Oxygen-dependent mechanisms include the enzymes NADPH oxidase and myeloperoxidase (MPO) that work together in killing bacteria (Figure 13.8). Overall, the MPO system is the most potent of the bactericidal mechanisms. An invading bacterium is recognized by the immune system and attacked by antibodies that bind it to a receptor on a phagocytic cell. After internalization of the microorganism has occurred, NADPH oxidase, located in the leukocyte cell membrane, is activated and reduces O_2 from the surrounding tissue to superoxide ($O_2^{\bar{\bullet}}$), a free radical, as NADPH is oxidized. The rapid consumption of O_2 that accompanies formation of $O_2^{\bar{\bullet}}$ is referred to as the "respiratory burst." [Note: Active NADPH oxidase is a membrane-associated complex containing a flavocytochrome plus additional peptides that translocate from the cytoplasm upon activation of the leukocyte. Electrons move from NADPH to O_2 via flavin adenine nucleotide (FAD) and heme, generating $O_2^{\bar{\bullet}}$. Rare genetic deficiencies in NADPH oxidase cause chronic granulomatous disease (CGD) characterized by severe, persistent infections and the formation of granulomas (nodular areas of inflammation) that sequester the bacteria that were not destroyed.] Next, $O_2^{\bar{\bullet}}$ is converted to H_2O_2 (a ROS), either spontaneously or catalyzed by superoxide dismutase. In the presence of MPO, a heme-containing lysosomal enzyme present within the phagolysosome, peroxide plus chloride ions are converted to hypochlorous acid ([HOCl] the major component of household bleach), which kills the bacteria. The peroxide can also be partially reduced to the hydroxyl radical (OH•), a ROS, or be fully reduced to water by catalase or glutathione peroxidase. [Note: Deficiencies in MPO do not confer increased susceptibility to infection because peroxide from NADPH oxidase is bactericidal.]

E. Synthesis of nitric oxide

Nitric oxide (NO) is recognized as a mediator in a broad array of biologic systems. NO is the endothelium-derived relaxing factor, which causes vasodilation by relaxing vascular smooth muscle. NO also acts as a neurotransmitter, prevents platelet aggregation, and plays an essential role in macrophage function. NO has a very short half-life in tissues (3–10 seconds) because it reacts with oxygen and superoxide and then is converted into nitrates and nitrites including peroxynitrite ($O{=}NOO^-$), a reactive nitrogen species (RNS). [Note: NO is a free radical gas that is often confused with nitrous oxide (N_2O), the "laughing gas" that is used as an anesthetic and is chemically stable.]

1. **Nitric oxide synthase:** Arginine, O_2, and NADPH are substrates for cytosolic NO synthase ([NOS] Figure 13.9). Flavin mononucleotide (FMN), FAD, heme, and tetrahydrobiopterin (see p. 268) are coenzymes, and NO and citrulline are products of the reaction. Three NOS, each the product of a different gene, have been identified. Two are constitutive (synthesized at a constant rate), Ca^{2+}–calmodulin-dependent enzymes (see p. 133). They are found primarily in endothelium (eNOS) and neural tissue (nNOS) and constantly produce very low levels of NO for vasodilation and neurotransmission. An inducible, Ca^{2+}-independent enzyme (iNOS) can be expressed in many cells, including macrophages and neutrophils, as an early defense against pathogens. The specific inducers for iNOS vary with cell type, and include proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ), and bacterial endotoxins such as lipopolysaccharide (LPS). These compounds promote synthesis of iNOS, which can result in large amounts of NO being produced over hours or even days.

2. **Actions of nitric oxide on vascular endothelium:** NO is an important mediator in the control of vascular smooth muscle tone. NO is synthesized by eNOS in endothelial cells and diffuses to vascular smooth muscle, where it activates the cytosolic form of guanylate cyclase (also known as guanylyl cyclase) to form cyclic guanosine monophosphate (cGMP). [Note: This reaction is analogous to the formation of cyclic AMP by adenylate cyclase (see p. 94), except that this guanylate cyclase is not membrane associated.] The resultant rise in cGMP causes activation of protein kinase G, which phosphorylates Ca^{2+} channels, causing decreased entry of Ca^{2+} into smooth muscle cells. This decreases the calcium–calmodulin activation of myosin light-chain kinase, thereby decreasing smooth muscle contraction and favoring relaxation. Vasodilator nitrates, such as nitroglycerin, are metabolized to NO, which causes relaxation of vascular smooth muscle and, therefore, lowers blood pressure. Thus, NO can be envisioned as an endogenous nitrovasodilator. [Note: NO is involved in penile erection. Sildenafil citrate, used in the treatment of erectile dysfunction, inhibits the phosphodiesterase that inactivates cGMP.]

3. **Role of nitric oxide in macrophage bactericidal activity:** In macrophages, iNOS activity is normally low, but synthesis of the enzyme is significantly stimulated by bacterial LPS and by release of IFN-γ and TNF-α in response to the infection. Activated macrophages form $O_2^{\overline{\bullet}}$ radicals (see p. 150) that combine with NO to form intermediates that decompose, producing the highly bactericidal OH• radical.

4. **Other functions of nitric oxide:** NO is a potent inhibitor of platelet adhesion and aggregation (by activating the cGMP pathway). It is also characterized as a neurotransmitter in the central and peripheral nervous systems.

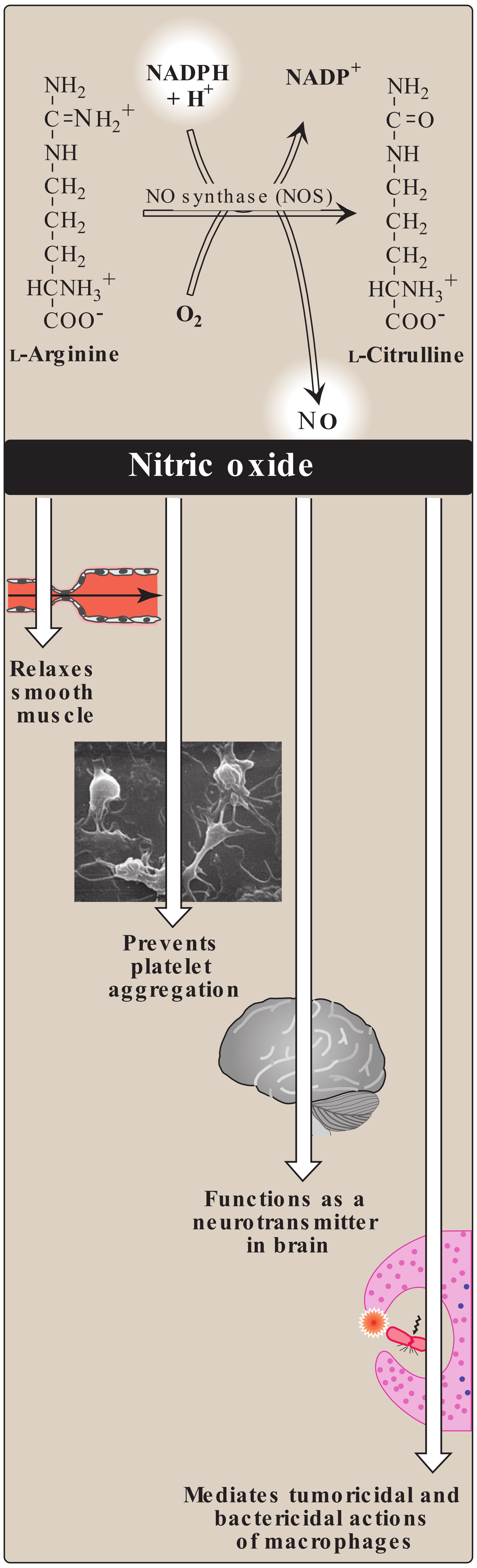


Figure 13.9
Synthesis and some of the actions of nitric oxide (NO). NADPH = reduced nicotinamide adenine dinucleotide phosphate. [Note: Flavin mononucleotide, flavin adenine dinucleotide, heme, and tetrahydrobiopterin are additional coenzymes required by NOS.]

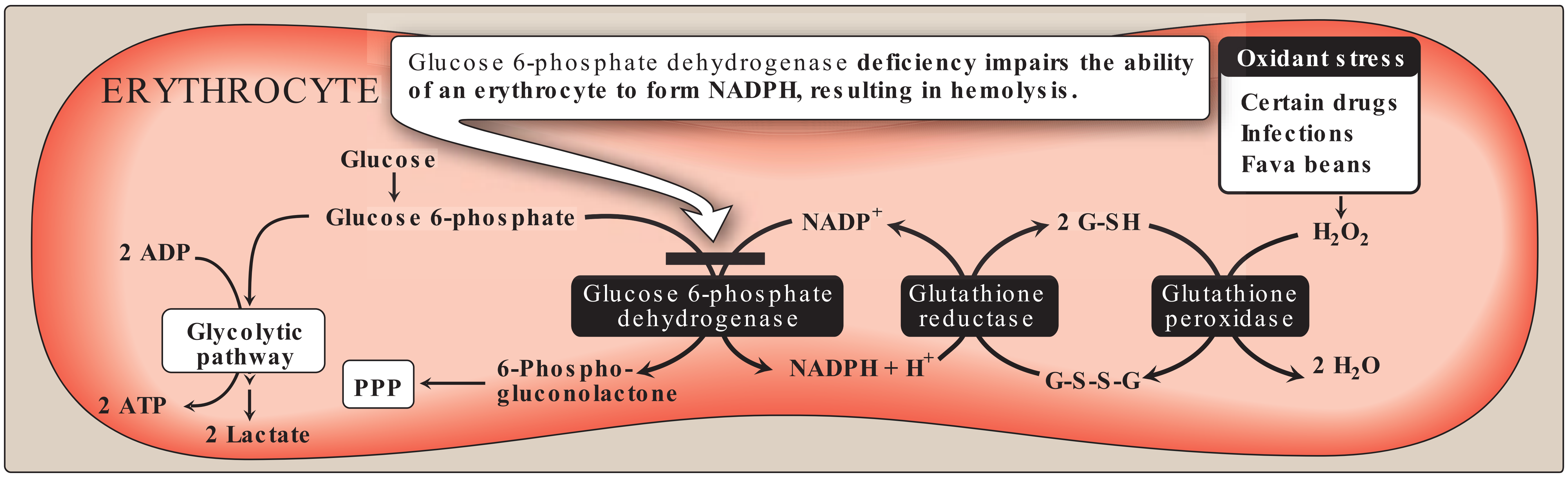

ERYTHROCYTE
Glucose 6-phosphate dehydrogenase **deficiency impairs the ability of an erythrocyte to form NADPH, resulting in hemolysis.**
Oxidant stress
Certain drugs
Infections
Fava beans
Glucose
Glucose 6-phosphate
2 ADP
Glycolytic pathway
2 ATP
2 Lactate
PPP
6-Phospho-gluconolactone
Glucose 6-phosphate dehydrogenase
NADP+
NADPH + H+
Glutathione reductase
2 G-SH
G-S-S-G
Glutathione peroxidase
H2O2
2 H2O

Figure 13.10
Pathways of glucose 6-phosphate metabolism in the erythrocyte. NADP(H) = nicotinamide adenine dinucleotide phosphate; G-SH = reduced glutathionine; G-S-S-G = oxidized glutathionine; PPP = pentose phosphate pathway.

V. GLUCOSE 6-PHOSPHATE DEHYDROGENASE DEFICIENCY

G6PD deficiency is a hereditary disease characterized by hemolytic anemia caused by the inability to detoxify oxidizing agents. G6PD deficiency is the most common disease-producing enzyme abnormality in humans, affecting more than 400 million individuals worldwide. This deficiency has the highest prevalence in the Middle East, tropical Africa and Asia, and parts of the Mediterranean. G6PD deficiency is X linked and is, in fact, a family of deficiencies caused by a number of different mutations in the gene coding for G6PD. Only some of the resulting protein variants cause clinical symptoms. [Note: In addition to hemolytic anemia, a clinical manifestation of G6PD deficiency is neonatal jaundice appearing 1–4 days after birth. The jaundice, which may be severe, typically results from increased production of unconjugated bilirubin (see p. 285).] The life span of individuals with a severe form of G6PD deficiency may be somewhat shortened as a result of complications arising from chronic hemolysis. This negative effect of G6PD deficiency has been balanced in evolution by an advantage in survival—an increased resistance to Plasmodium falciparum malaria. [Note: Sickle cell trait and β-thalassemia minor also confer resistance to malaria.]

A. Role of glucose 6-phosphate dehydrogenase in red blood cells

Diminished G6PD activity impairs the ability of the cell to form the NADPH that is essential for the maintenance of the G-SH pool. This results in a decrease in the cellular detoxification of free radicals and peroxides formed within the cell (Figure 13.10). G-SH also helps maintain the reduced states of sulfhydryl groups in proteins, including hemoglobin. Oxidation of those sulfhydryl groups leads to the formation of denatured proteins that form insoluble masses (called Heinz bodies) that attach to RBC membranes (Figure 13.11). Additional oxidation of membrane proteins causes RBCs to be rigid (less deformable), and they are removed from the circulation by macrophages in the spleen and liver. Although G6PD deficiency occurs in all cells of the affected individual, it is most severe in RBCs, where the pentose phosphate pathway provides the only means of generating NADPH.

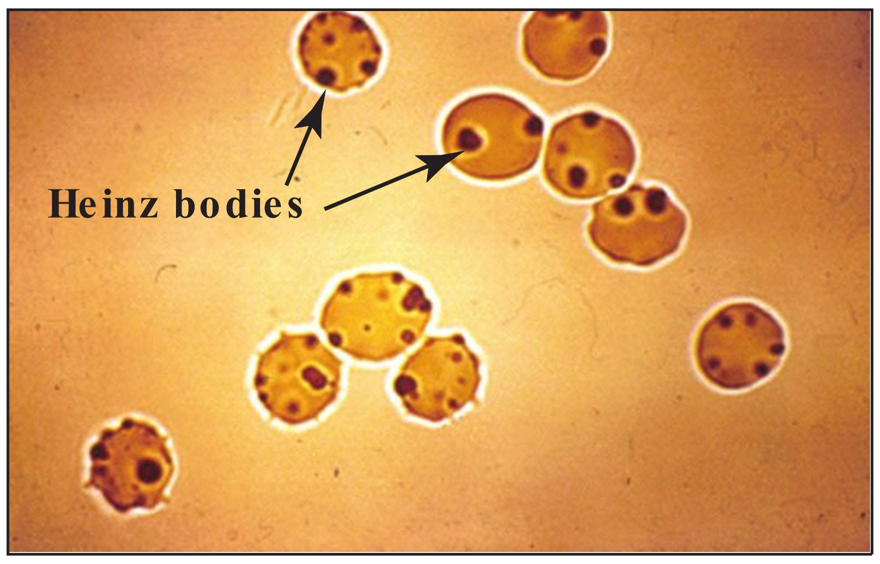

Heinz bodies

Figure 13.11
Heinz bodies in erythrocytes of a patient with glucose 6-phosphate dehydrogenase deficiency.

Other tissues have alternative sources for NADPH production (such as NADP$^+$-dependent malate dehydrogenase [malic enzyme]; see p. 186) that can keep G-SH reduced. The RBC has no nucleus or ribosomes and cannot renew its supply of the enzyme. Thus, RBCs are particularly vulnerable to enzyme variants with diminished stability.

B. Precipitating factors in glucose 6-phosphate dehydrogenase deficiency

Most individuals who have inherited one of the G6PD mutations do not show clinical manifestations (that is, they are asymptomatic). However, some patients with G6PD deficiency develop hemolytic anemia if they are treated with an oxidant drug, ingest fava beans, or contract a severe infection.

1. **Oxidant drugs:** Commonly used drugs that produce hemolytic anemia in patients with G6PD deficiency are best remembered from the mnemonic AAA: antibiotics (for example, sulfamethoxazole and chloramphenicol), antimalarials (for example, primaquine but not chloroquine or quinine), and antipyretics (for example, acetanilid but not acetaminophen).

2. **Favism:** Some forms of G6PD deficiency, for example the Mediterranean variant, are particularly susceptible to the hemolytic effect of the fava (broad) bean, a dietary staple in the Mediterranean region. Favism, the hemolytic effect of ingesting fava beans, is not observed in all individuals with G6PD deficiency, but all patients with favism have G6PD deficiency.

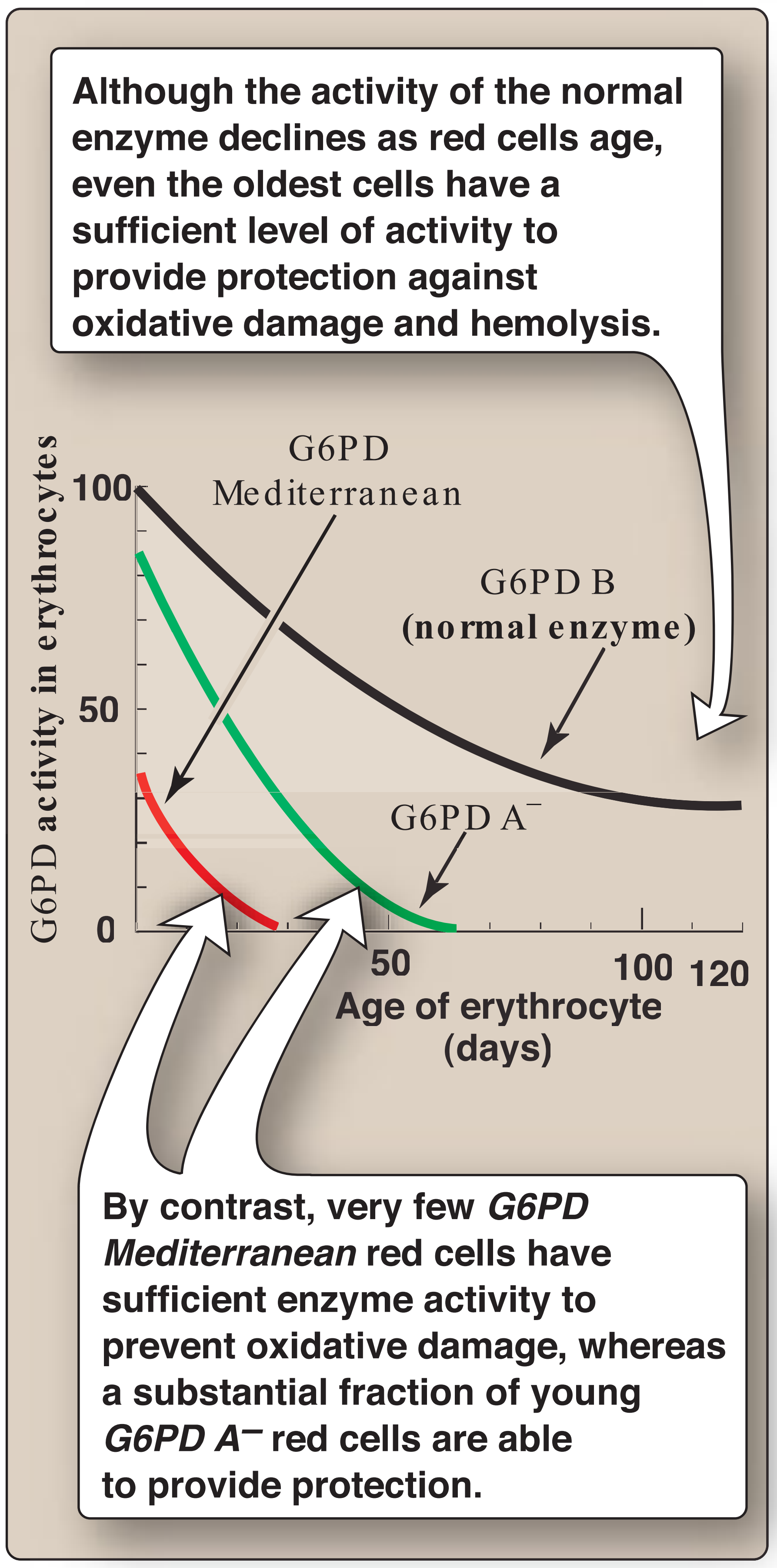
3. **Infection:** Infection is the most common precipitating factor of hemolysis in G6PD deficiency. The inflammatory response to infection results in the generation of free radicals in macrophages, which can diffuse into the RBC and cause oxidative damage.

C. Properties of the variant enzymes

Almost all G6PD variants are caused by point mutations (see p. 433) in the gene for G6PD. Some mutations do not disrupt the structure of the enzyme's active site and, therefore, do not affect enzymic activity. However, many mutant enzymes show altered kinetic properties. For example, variant enzymes may show decreased catalytic activity, decreased stability, or an alteration of binding affinity for NADP$^+$, NADPH, or glucose 6-phosphate. The severity of the disease usually correlates with the amount of residual enzyme activity in the patient's RBC. For example, variants can be classified as shown in Figure 13.12. G6PD A$^-$ is the prototype of the moderate (Class III) form of the disease. The RBCs contain an unstable but kinetically normal G6PD, with most of the enzyme activity present in the reticulocytes and younger RBCs (Figure 13.13). The oldest RBCs, therefore, have the lowest level of enzyme activity and are preferentially removed in a hemolytic episode. G6PD Mediterranean is the prototype of a more severe (Class II) deficiency in which the enzyme has decreased stability resulting in decreased enzymic activity. Class I mutations (rare) are the most severe and are associated with chronic nonspherocytic hemolytic anemia, which occurs even in the absence of oxidative stress.

Class	Clinical symptoms	Residual enzyme activity
I	Very severe (chronic hemolytic anemia)	<10%
II	Severe (episodic hemolytic anemia)	<10%
III	Moderate	10%–60%
IV	None	>60%

Figure 13.12
Classification of glucose 6-phosphate dehydrogenase (G6PD) deficiency variants. Note: Class V variants (not shown in table) result in overproduction of G6PD.



Although the activity of the normal enzyme declines as red cells age, even the oldest cells have a sufficient level of activity to provide protection against oxidative damage and hemolysis.

By contrast, very few *G6PD Mediterranean* red cells have sufficient enzyme activity to prevent oxidative damage, whereas a substantial fraction of young *G6PD A$^-$* red cells are able to provide protection.

Figure 13.13
Decline of erythrocyte glucose 6-phosphate dehydrogenase (G6PD) activity with cell age for the three most commonly encountered forms of the enzyme.

D. Molecular biology of glucose 6-phosphate dehydrogenase

The cloning of the gene for G6PD and the sequencing of its DNA (see p. 467) have permitted the identification of mutations that cause G6PD deficiency. More than 400 different G6PD variants have been identified, a finding that explains the numerous biochemical and clinical phenotypes that have been described. Most mutations that result in enzymic deficiency are missense mutations (see p. 433) in the coding region. Both G6PD A^{-} and G6PD Mediterranean represent mutant enzymes that differ from the respective normal variants by a single amino acid. Large deletions or frameshift mutations have not been identified, suggesting that complete absence of G6PD activity is probably lethal.

VI. CHAPTER SUMMARY

The pentose phosphate pathway includes two irreversible oxidative reactions followed by a series of reversible sugar–phosphate interconversions (Figure 13.14). No ATP is directly consumed or produced in the cycle. The reduced nicotinamide adenine dinucleotide phosphate (NADPH)-producing **oxidative portion** of the pentose phosphate pathway is important in providing reducing equivalents for reductive biosynthesis and detoxification reactions. In this part of the pathway, **glucose 6-phosphate** is irreversibly converted to **ribulose 5-phosphate**, and **two NADPH** are produced. The regulated step is catalyzed by glucose 6-phosphate dehydrogenase (G6PD), which is strongly **inhibited by NADPH**. **Reversible nonoxidative reactions** interconvert sugars. This part of the pathway is the **source of ribose 5-phosphate**, required for nucleotide and nucleic acid synthesis. Because the reactions are reversible, they can be entered from fructose 6-phosphate and glyceraldehyde 3-phosphate (glycolytic intermediates) if ribose is needed and G6PD is inhibited. **NADPH** is a source of reducing equivalents in **reductive biosynthesis**, such as the production of fatty acids in liver, adipose tissue, and the mammary gland, and steroid hormones in the placenta, ovaries, testes, and adrenal cortex. It is also required by red blood cells (RBCs) for the **reduction of hydrogen peroxide**, providing the reducing equivalents required by **glutathione (GSH)**. GSH is used by glutathione peroxidase to reduce peroxide to water. The oxidized glutathione (GSSH) produced is reduced by glutathione reductase, using NADPH as the source of electrons. NADPH provides reducing equivalents for the mitochondrial cytochrome P450 monooxygenase **system**, which is used in **steroid hormone synthesis** in steroidogenic tissue, **bile acid synthesis** in liver, and **vitamin D activation in the liver and kidney**. The microsomal system uses NADPH to **detoxify** foreign compounds (xenobiotics), such as drugs and a variety of pollutants. NADPH provides the reducing equivalents for phagocytes in the process of eliminating invading microorganisms. NADPH oxidase uses molecular oxygen and electrons from NADPH to produce **superoxide radicals**, which, in turn, can be converted to peroxide by superoxide dismutase. Myeloperoxidase catalyzes the formation of bactericidal hypochlorous acid from peroxide and chloride ions. Rare genetic defects in NADPH oxidase cause **chronic granulomatous disease** characterized by severe, persistent, infections and formation of granulomas. NADPH is required for the synthesis of **nitric oxide (NO)**, an important free radical gas that causes **vasodilation** by relaxing vascular smooth muscle, acts as a **neurotransmitter**, prevents **platelet aggregation**, and helps mediate **macrophage bactericidal activity**. NO is made from arginine and O_2 by three different NADPH-dependent NO synthases (NOS). The endothelial (eNOS), and neuronal (nNOS) isozymes constantly produce very low levels of NO for vasodilation and neurotransmission, respectively. The inducible isozyme (iNOS) produces large amounts of NO for defense against pathogens. G6PD **deficiency** impairs the ability of the cell to form the NADPH that is essential for the maintenance of the GSH pool. The cells most affected are the **RBCs** because they do not have additional sources of NADPH. G6PD deficiency is an **X-linked disease** characterized by **hemolytic anemia** caused by the production of free radicals and peroxides following administration of **oxidant drugs**, ingestion of **fava beans**, or severe **infections**. The extent of the anemia depends on the amount of residual enzyme. Class I variants, the most severe (and least common), are associated with **chronic nonspherocytic hemolytic anemia**. Babies with G6PD deficiency may experience **neonatal jaundice**.

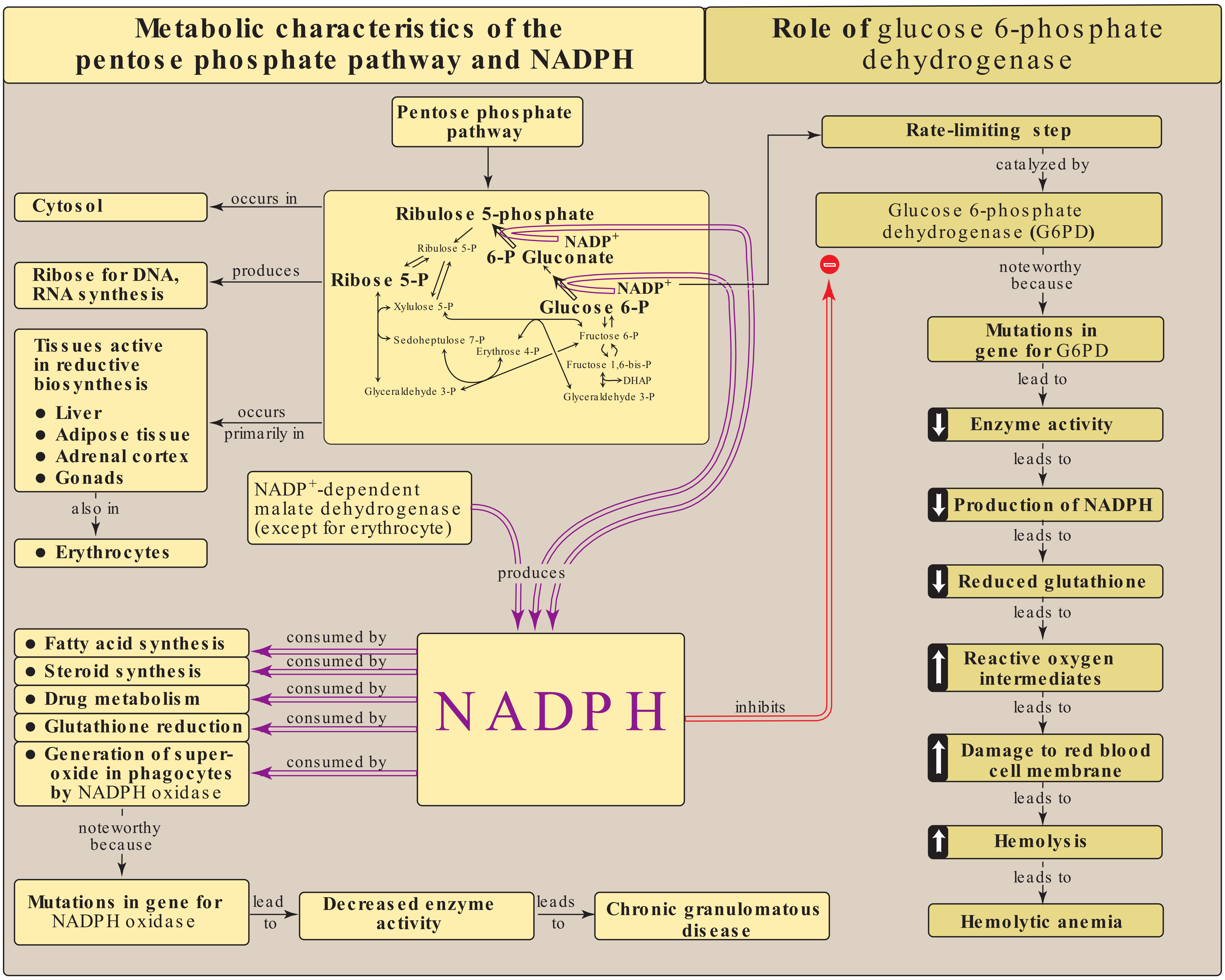

Metabolic characteristics of the pentose phosphate pathway and NADPH
Role of glucose 6-phosphate dehydrogenase
Pentose phosphate pathway
Cytosol
occurs in
Ribose for DNA, RNA synthesis
produces
Tissues active in reductive biosynthesis
● Liver
● Adipose tissue
● Adrenal cortex
● Gonads
occurs primarily in
also in
● Erythrocytes
Ribulose 5-phosphate
NADP+
6-P Gluconate
Ribulose 5-P
Ribose 5-P
NADP+
Glucose 6-P
Xylulose 5-P
Sedoheptulose 7-P
Erythrose 4-P
Fructose 6-P
Fructose 1,6-bis-P
DHAP
Glyceraldehyde 3-P
Glyceraldehyde 3-P
NADP+-dependent malate dehydrogenase (except for erythrocyte)
produces
NADPH
consumed by
● Fatty acid synthesis
consumed by
● Steroid synthesis
consumed by
● Drug metabolism
consumed by
● Glutathione reduction
consumed by
● Generation of superoxide in phagocytes by NADPH oxidase
noteworthy because
Mutations in gene for NADPH oxidase
lead to
Decreased enzyme activity
leads to
Chronic granulomatous disease
inhibits
Rate-limiting step
catalyzed by
Glucose 6-phosphate dehydrogenase (G6PD)
noteworthy because
Mutations in gene for G6PD
lead to
Enzyme activity
leads to
Production of NADPH
leads to
Reduced glutathione
leads to
Reactive oxygen intermediates
leads to
Damage to red blood cell membrane
leads to
Hemolysis
leads to
Hemolytic anemia

Figure 13.14
Key concept map for the pentose phosphate pathway and nicotinamide adenine dinucleotide phosphate (NADPH).

Glycosaminoglycans, Proteoglycans, and Glycoproteins

14

I. OVERVIEW OF GLYCOSAMINOGLYCANS

Glycosaminoglycans (GAGs) are large complexes of negatively charged heteropolysaccharide chains. They are generally associated with a small amount of protein ("core protein"), forming proteoglycans, which typically consist of up to 95% carbohydrate. [Note: This is in comparison to the glycoproteins, which consist primarily of protein with a variable (but typically small) amount of carbohydrate (see p. 165).] GAGs have the special ability to bind large amounts of water, thereby producing the gel-like matrix that forms the basis of the body's ground substance, which, along with fibrous structural proteins such as collagen, elastin, and fibrillin-1, and adhesive proteins such as fibronectin, make up the extracellular matrix (ECM). The hydrated GAGs serve as a flexible support for the ECM, interacting with the structural and adhesive proteins, and as a molecular sieve, influencing movement of materials through the ECM. The viscous, lubricating properties of mucous secretions also result from the presence of GAGs, which led to the original naming of these compounds as mucopolysaccharides.

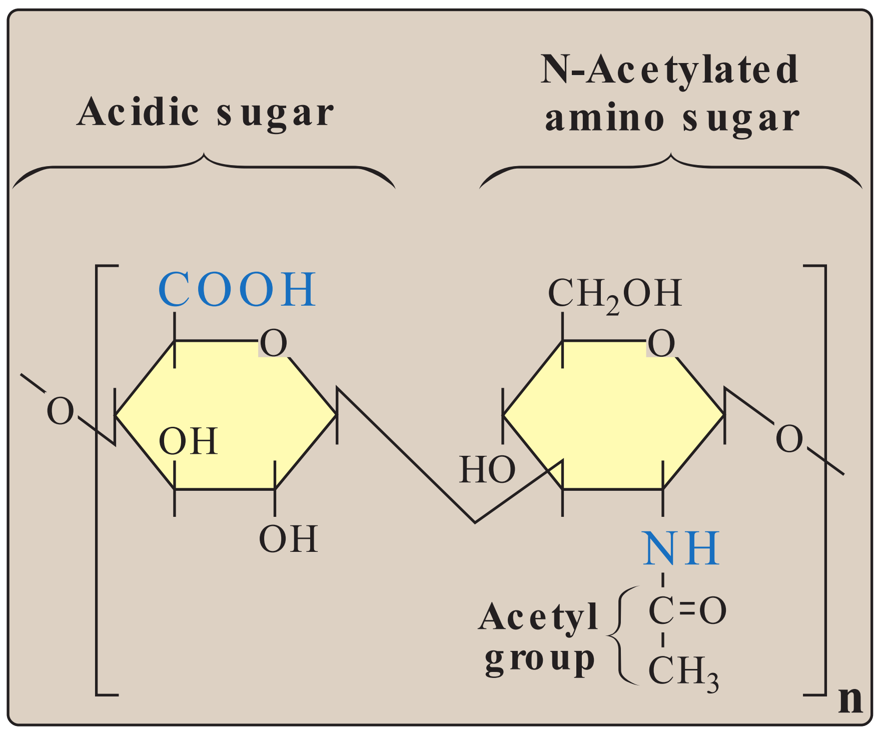


Figure 14.1
Repeating disaccharide unit.

II. STRUCTURE OF GLYCOSAMINOGLYCANS

GAGs are long, unbranched, heteropolysaccharide chains composed of a repeating disaccharide unit [acidic sugar–amino sugar]$_n$ (Figure 14.1). [Note: A single exception is keratan sulfate, which contains galactose rather than an acidic sugar.] The amino sugar is either D-glucosamine or D-galactosamine, in which the amino group is usually acetylated, thus eliminating its positive charge. The amino sugar may also be sulfated on carbon 4 or 6 or on a nonacetylated nitrogen. The acidic sugar is either D-glucuronic acid or its C-5 epimer L-iduronic acid (Figure 14.2). These acidic sugars contain carboxyl groups that are negatively charged at physiologic pH and, together with the sulfate groups, give GAGs their strongly negative nature.

A. Relationship between glycosaminoglycan structure and function

Because of their large number of negative charges, these heteropolysaccharide chains tend to be extended in solution. They repel each other and are surrounded by a shell of water molecules. When brought together, they "slide" past each other, much as two magnets with the same polarity seem to slide past each other. This produces

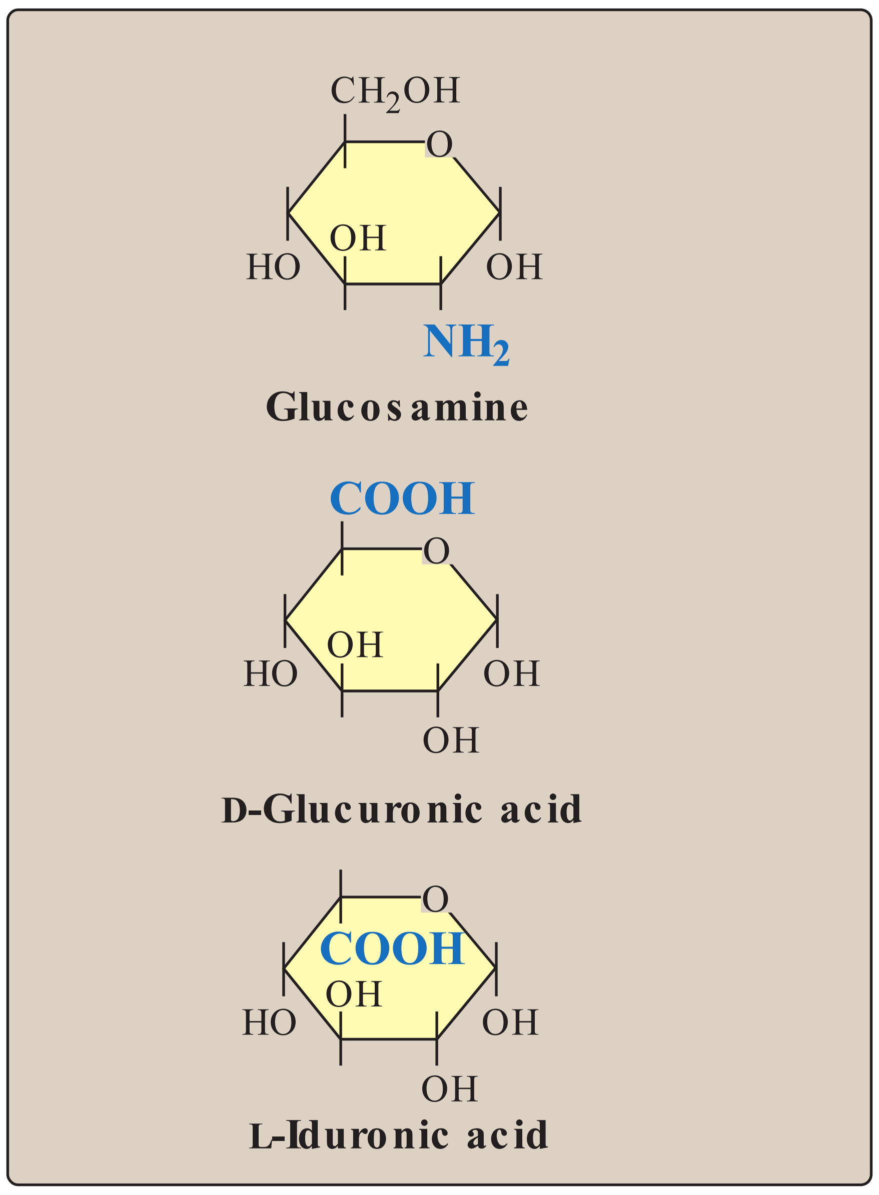


Figure 14.2
Some monosaccharide units found in glycosaminoglycans.

the "slippery" consistency of mucous secretions and synovial fluid. When a solution of GAGs is compressed, the water is "squeezed out," and the GAGs are forced to occupy a smaller volume. When the compression is released, the GAGs spring back to their original, hydrated volume because of the repulsion of their negative charges. This property contributes to the resilience of synovial fluid and the vitreous humor of the eye (Figure 14.3).

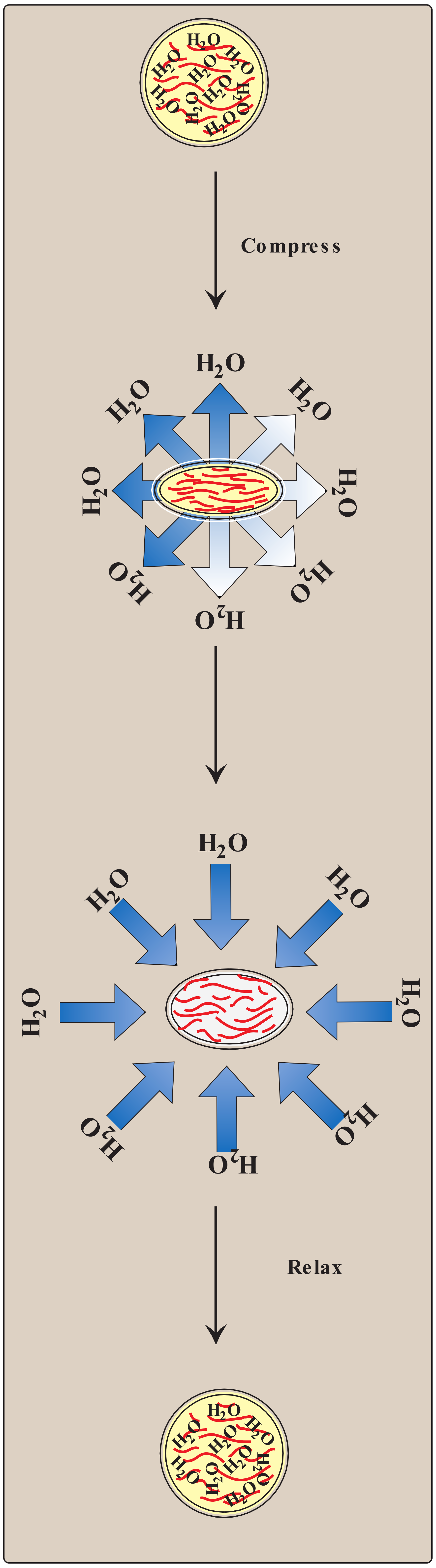


Figure 14.3
Resilience of glycosaminoglycans.

B. Classification of the glycosaminoglycans

The six major types of glycosaminoglycans are divided according to monomeric composition, type of glycosidic linkages, and degree and location of sulfate units. The structure of the GAGs and their distribution in the body is illustrated in Figure 14.4. All GAGs, except for hyaluronic acid, are sulfated and are found covalently attached to protein, forming proteoglycan monomers.

C. Proteoglycans

Proteoglycans are found in the ECM and on the outer surface of cells.

1. **Structure of proteoglycan monomers:** A proteoglycan monomer found in cartilage consists of a core protein to which up to 100 linear chains of GAGs are covalently attached. These chains, which may each be composed of up to 200 disaccharide units, extend out from the core protein and remain separated from each other because of charge repulsion. The resulting structure resembles a "bottle brush" (Figure 14.5). In cartilage proteoglycan, the species of GAGs include chondroitin sulfate and keratan sulfate. [Note: Proteoglycans are grouped into gene families that encode core proteins with common structural features. The aggrecan family (aggrecan, versecan, neurocan, and brevican), abundant in cartilage, is an example.]

2. **Linkage between the carbohydrate chain and the protein:** This linkage is most commonly through a trihexoside (galactose-galactose-xylose) and a serine residue, respectively. An O-glycosidic bond is formed between the xylose and the hydroxyl group of the serine (Figure 14.6).

3. **Proteoglycan aggregates:** The proteoglycan monomers associate with a molecule of hyaluronic acid to form proteoglycan aggregates.The association is not covalent but occurs primarily through ionic interactions between the core protein and the hyaluronic acid. The association is stabilized by additional small proteins called link proteins (Figure 14.7).

III. SYNTHESIS OF GLYCOSAMINOGLYCANS

The heteropolysaccharide chains are elongated by the sequential addition of alternating acidic and amino sugars donated by their uridine diphosphate (UDP)-derivatives. The reactions are catalyzed by a family of specific glycosyltransferases. The synthesis of GAGs is analogous to that of glycogen (see p. 126) except that GAGs are produced for export from the cell. Their synthesis occurs, therefore, primarily in the Golgi, rather than in the cytosol.

CHONDROITIN 4- AND 6-SULFATES

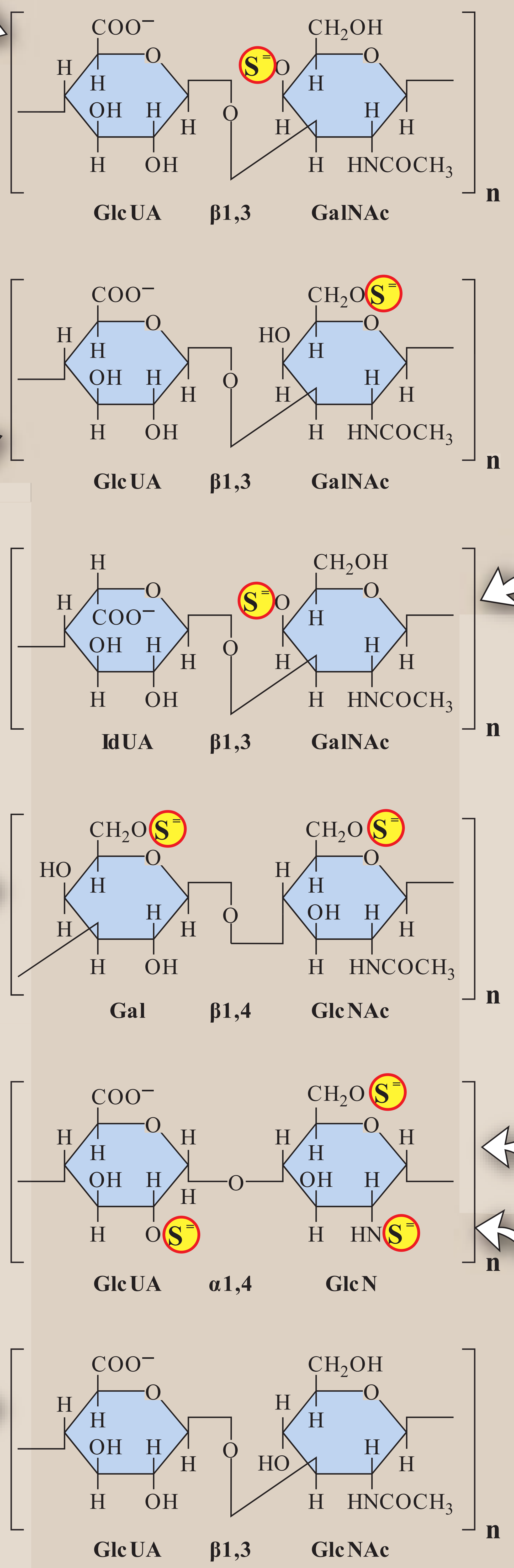
- Disaccharide unit:
  N-acetylgalactosamine with sulfate on either C 4 or C 6 and glucuronic acid
- Most abundant GAGs in the body
- Found in cartilage, tendons, ligaments, and aorta
- Form proteoglycan aggregates, often aggregating noncovalently with hyaluronic acid
- In cartilage, bind collagen and hold fibers in a tight, strong network

KERATAN SULFATES (KS) I and II

- Disaccharide unit:
  N-acetylglucosamine and galactose (no uronic acid); variable sulfate content may be present on C 6 of either sugar
- Most heterogeneous GAGs because they contain additional monosaccharides such as L-fucose, N-acetylneuraminic acid, and mannose
- KS I found in corneas; KS II found in loose connective tissue proteoglycan aggregates with chondroitin sulfate

HYALURONIC ACID (HYALURONATE)

- Disaccharide unit:
  N-acetylglucosamine and glucuronic acid
- Different from other GAGs: unsulfated, not covalently attached to protein, and only GAG not limited to animal tissue, but also found in bacteria
- Serves as a lubricant and shock absorber
- Found in synovial fluid of joints, vitreous humor of the eye, the umbilical cord, loose connective tissue, and cartilage



DERMATAN SULFATE

- Disaccharide unit:
  N-acetylgalactosamine and L-iduronic acid (with variable amounts of glucuronic acid)
- Found in skin, blood vessels, and heart valves

HEPARIN

- Disaccharide unit:
  Glucosamine and glucuronic or iduronic acid; most glucosamine residues are bound in sulfamide linkages; sulfate also found on C 3 or C 6 of glucosamine and C 2 of uronic acid (an average of 2.5 S per disaccharide unit)
- α-Linkage joins the sugars
- Unlike other GAGs that are extracellular compounds, heparin is an intracellular component of mast cells that line arteries, especially in liver, lungs, and skin
- Serves as an anticoagulant

HEPARAN SULFATE

- Disaccharide unit:
  Same as heparin except some glucosamines are acetylated, and there are fewer sulfate groups
- Extracellular GAG found in basement membrane and as a ubiquitous component of cell surfaces

Figure 14.4
Structure and distribution of glycosaminoglycans (GAGs). Sulfate groups (S) are shown in all possible positions. GlcUA = glucuronic acid; IdUA = iduronic acid; GalNAc = N-acetylgalactosamine; GlcNAc = N-acetylglucosamine; GlcN = glucosamine; Gal = galactose.

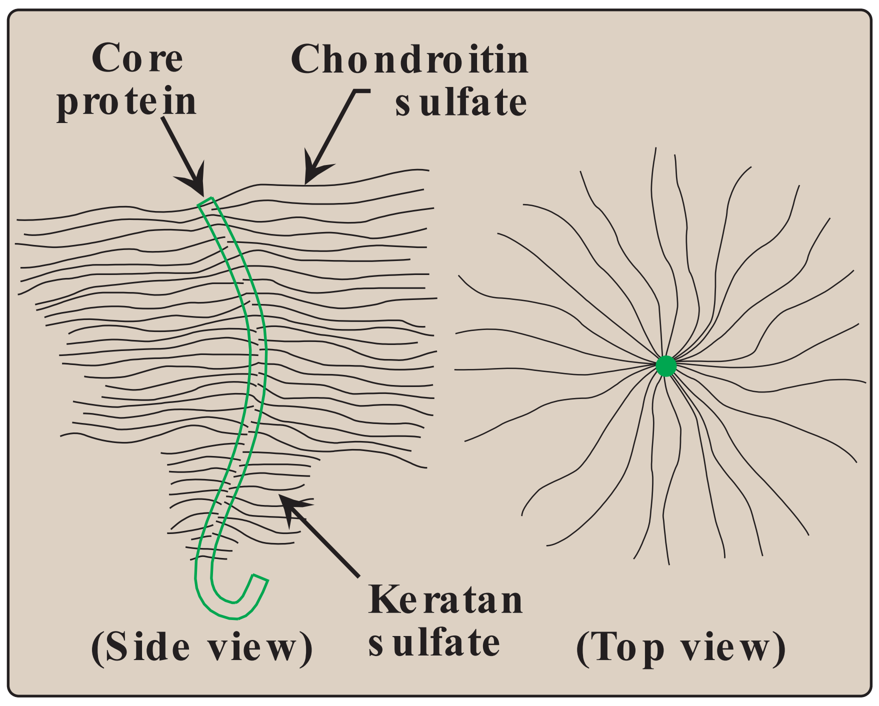

Core protein
Chondroitin sulfate
Keratan sulfate
(Side view)
(Top view)

Figure 14.5
"Bottle-brush" model of a cartilage proteoglycan monomer.

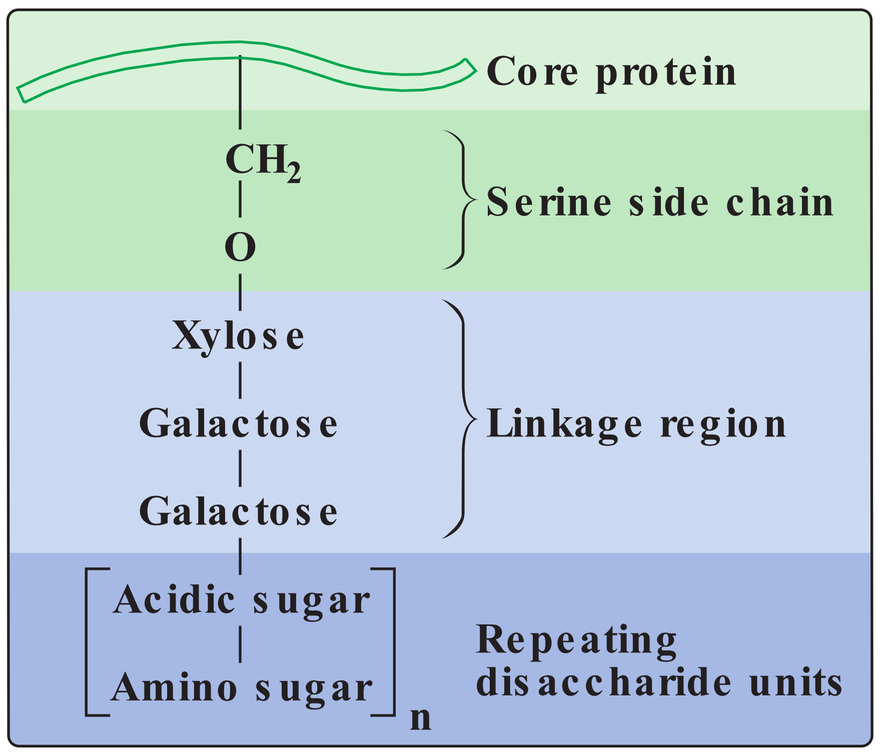

Core protein
CH₂
O
Serine side chain
Xylose
Galactose
Galactose
Linkage region
Acidic sugar
Amino sugar
n
Repeating disaccharide units

Figure 14.6
Linkage region of glycosaminoglycans.

A. Synthesis of amino sugars

Amino sugars are essential components of GAGs, glycoproteins, and glycolipids and are also found in some antibiotics. The synthetic pathway of amino sugars is very active in connective tissues, where as much as 20% of glucose flows through this pathway.

1. **N-Acetylglucosamine and N-acetylgalactosamine:** The monosaccharide fructose 6-phosphate is the precursor of N-acetylglucosamine (GlcNAc), N-acetylgalactosamine (GalNAc), and the sialic acids, including N-acetylneuraminic acid (NANA). In each of these sugars, a hydroxyl group of the precursor is replaced by an amino group donated by glutamine (Figure 14.8). [Note: The amino groups are then almost always acetylated.] The UDP-derivatives of GlcNAc and GalNAc are synthesized by reactions analogous to those described for UDP-glucose synthesis (see p. 126). These nucleotide sugars are the activated forms of the monosaccharides that can be used to elongate the carbohydrate chains.

2. **N-Acetylneuraminic acid:** NANA, a nine-carbon, acidic monosaccharide, is a member of the family of sialic acids, each of which is acylated at a different site. These compounds are usually found as terminal carbohydrate residues of oligosaccharide side chains of glycoproteins; glycolipids; or, less frequently, of GAGs. The carbons and nitrogens in NANA come from N-acetylmannosamine and phosphoenolpyruvate (an intermediate in the glycolytic pathway; see p. 102). [Note: Before NANA can be added to a growing oligosaccharide, it must be converted into its active form by reacting with cytidine triphosphate (CTP). The enzyme CMP-NANA synthetase catalyzes the reaction. This is the only nucleotide sugar in human metabolism in which the carrier nucleotide is a monophosphate.]

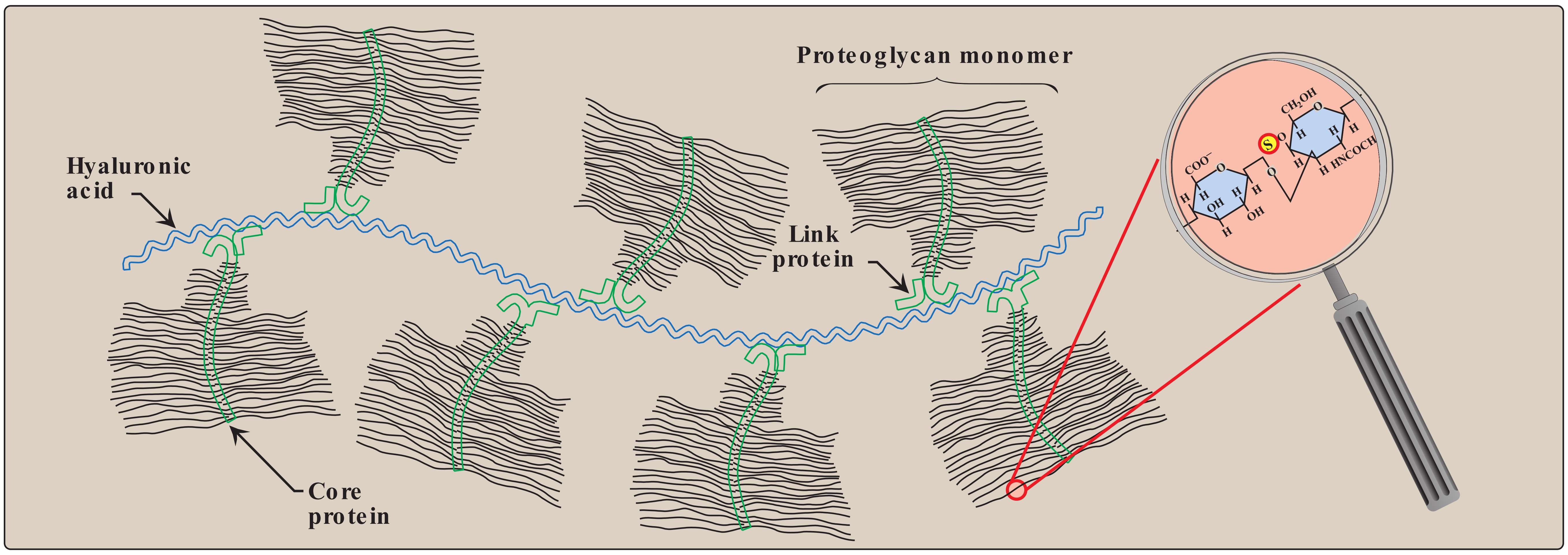

Proteoglycan monomer
Hyaluronic acid
Link protein
Core protein
CH₂OH
COO⁻
S
HNCOCH
OH

Figure 14.7
Proteoglycan aggregate.

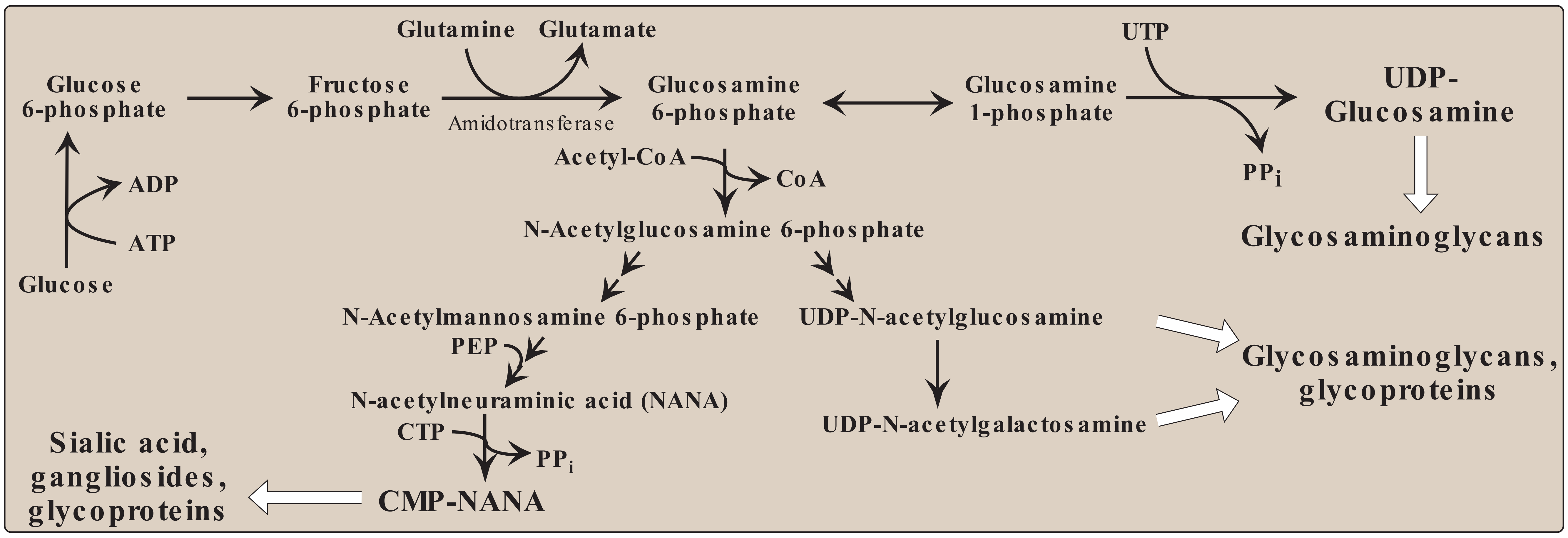

Glucose 6-phosphate
Glucose
ATP
ADP
Fructose 6-phosphate
Glutamine
Glutamate
Amidotransferase
Glucosamine 6-phosphate
Glucosamine 1-phosphate
UTP
PP$_i$
UDP-Glucosamine
Glycosaminoglycans
Acetyl-CoA
CoA
N-Acetylglucosamine 6-phosphate
N-Acetylmannosamine 6-phosphate
PEP
N-acetylneuraminic acid (NANA)
CTP
PP$_i$
CMP-NANA
Sialic acid, gangliosides, glycoproteins
UDP-N-acetylglucosamine
UDP-N-acetylgalactosamine
Glycosaminoglycans, glycoproteins

Figure 14.8
Synthesis of the amino sugars. UTP = uridine triphosphate; UDP = uridine diphosphate; CoA = coenzyme A; PEP = phosphoenoylpyruvate; CTP = cytidine triphosphate; CMP = cytidine monophosphate; PP_i = pyrophosphate.

B. Synthesis of acidic sugars

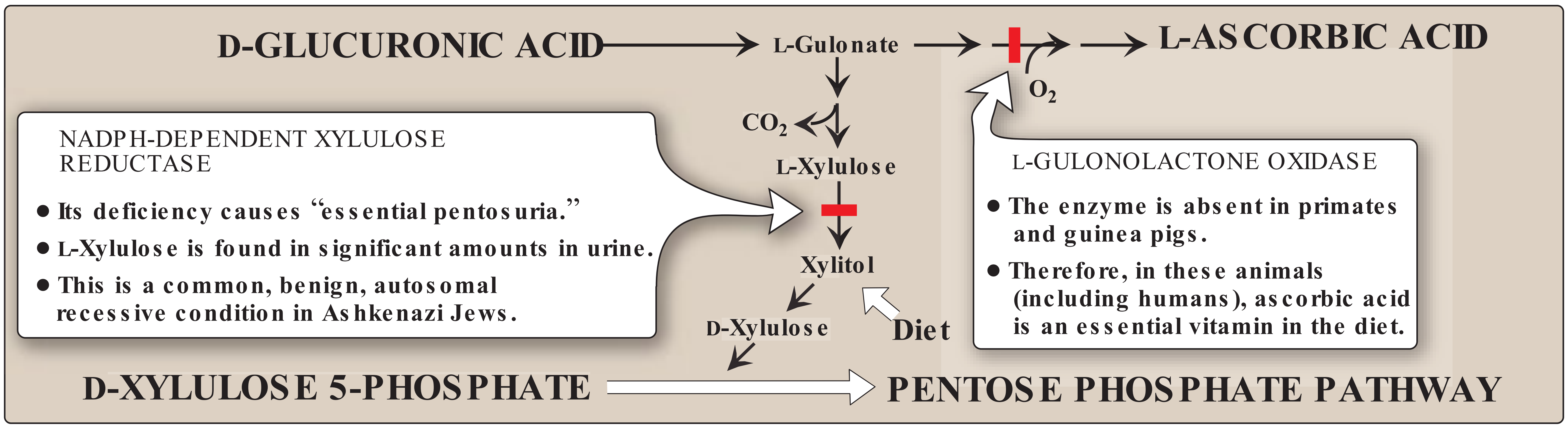
D-Glucuronic acid, whose structure is that of glucose with an oxidized carbon 6 (–CH_2OH → –COOH) and its C-5 epimer, L-iduronic acid, are essential components of GAGs. Glucuronic acid is also required in detoxification reactions of a number of insoluble compounds, such as bilirubin (see p. 282), steroids, and many drugs, including the statins (see p. 224). In plants and mammals (other than guinea pigs and primates, including humans), glucuronic acid serves as a precursor of ascorbic acid (vitamin C). The uronic acid pathway also provides a mechanism by which dietary D-xylulose can enter the central metabolic pathways.

1. **Glucuronic acid:** Glucuronic acid can be obtained in small amounts from the diet. It can also be obtained from the lysosomal degradation of GAGs, or from glucose 6-phosphate via the uronic acid pathway. The end product of glucuronic acid metabolism in humans is D-xylulose 5-phosphate, which can enter the pentose phosphate pathway and produce the glycolytic intermediates glyceraldehyde 3-phosphate and fructose 6-phosphate (Figure 14.9; see also Figure 13.2, p. 146). The active form of glucuronic acid that donates the sugar in GAG synthesis and other glucuronidation reactions is UDP-glucuronic acid, which is produced by oxidation of UDP-glucose (Figure 14.10).

2. **L-Iduronic acid synthesis:** Synthesis of L-iduronic acid residues occurs after D-glucuronic acid has been incorporated into the carbohydrate chain. Uronosyl 5-epimerase causes epimerization of the D- to the L-sugar.

C. Synthesis of the core protein

The core protein is synthesized by ribosomes on the rough endoplasmic reticulum (RER) and enters the RER lumen. The protein is then glycosylated by membrane-bound glycosyltransferases located in the Golgi.


D-GLUCURONIC ACID
L-Gulonate
L-ASCORBIC ACID
O_2
CO_2
L-Xylulose
Xylitol
D-Xylulose
Diet
D-XYLULOSE 5-PHOSPHATE
PENTOSE PHOSPHATE PATHWAY

NADPH-DEPENDENT XYLULOSE REDUCTASE

- Its deficiency causes "essential pentosuria."
- L-Xylulose is found in significant amounts in urine.
- This is a common, benign, autosomal recessive condition in Ashkenazi Jews.

L-GULONOLACTONE OXIDASE

- The enzyme is absent in primates and guinea pigs.
- Therefore, in these animals (including humans), ascorbic acid is an essential vitamin in the diet.

Figure 14.9
Metabolism of glucuronic acid. NADPH = reduced nicotinamide adenine dinucleotide phosphate.

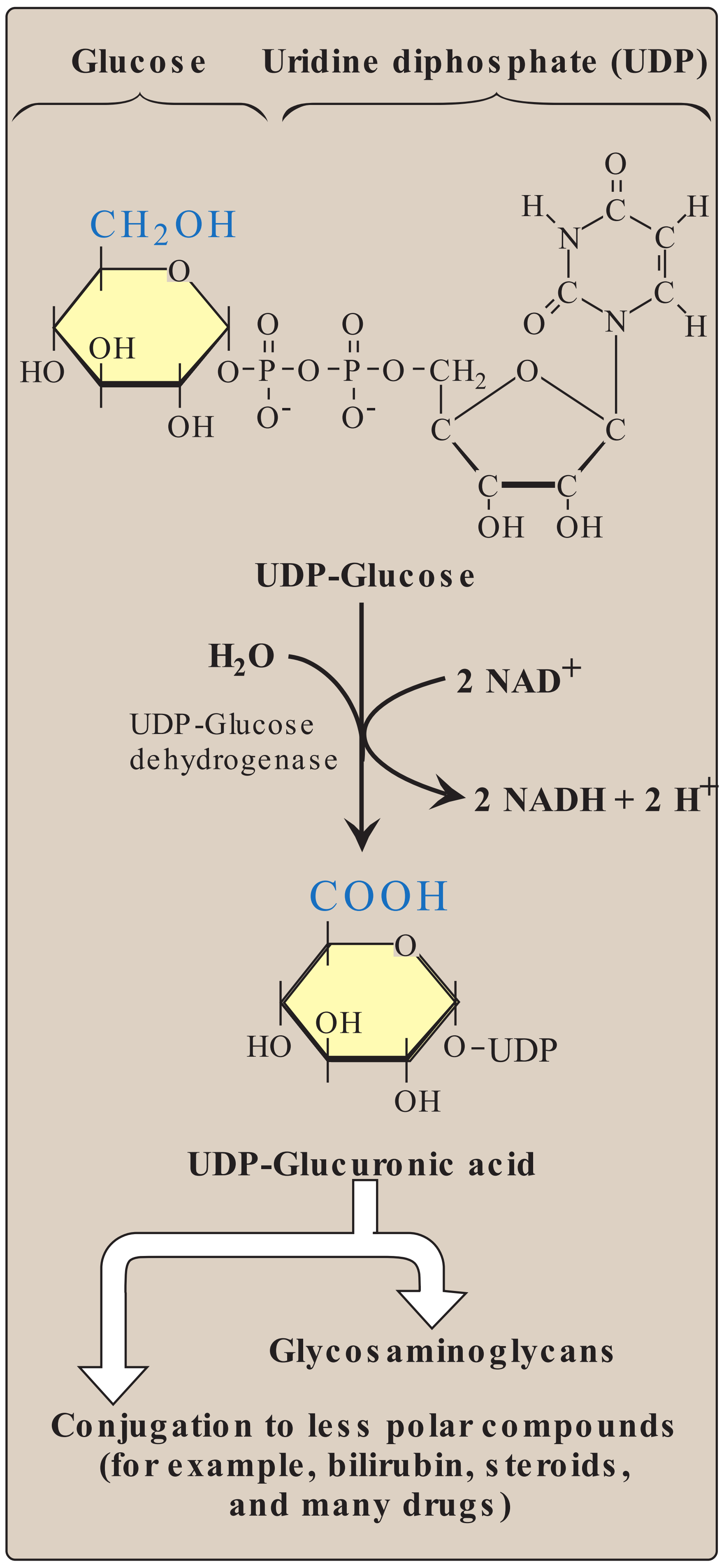

Glucose
Uridine diphosphate (UDP)
CH_2OH
O
OH
HO
OH
O
O
O-P-O-P-O-CH_2
O^-
O^-
O
H
N
C
H
O
C
N
H
OH
OH
UDP-Glucose
H_2O
2 NAD^+
UDP-Glucose dehydrogenase
2 NADH + 2 H^+
COOH
O
OH
HO
O-UDP
OH
UDP-Glucuronic acid
Glycosaminoglycans
Conjugation to less polar compounds (for example, bilirubin, steroids, and many drugs)

Figure 14.10
Oxidation of UDP-glucose to UDP-glucuronic acid. NAD(H) = nicotinamide adenine dinucleotide.

D. Synthesis of the carbohydrate chain

Carbohydrate chain formation begins by synthesis of a short linkage region on the core protein on which carbohydrate chain synthesis will be initiated. The most common linkage region is formed by the transfer of a xylose from UDP-xylose to the hydroxyl group of a serine (or threonine) catalyzed by xylosyltransferase. Two galactose molecules are then added, completing the trihexoside. This is followed by sequential addition of alternating acidic and amino sugars (Figure 14.11) and epimerization of some D-glucuronyl to L-iduronyl residues.

E. Addition of sulfate groups

Sulfation of a GAG occurs after the particular monosaccharide to be sulfated has been incorporated into the growing carbohydrate chain. The source of the sulfate is 3'-phosphoadenosyl-5'-phosphosulfate ([PAPS], a molecule of adenosine monophosphate with a sulfate group attached to the 5'-phosphate). The sulfation reaction is catalyzed by sulfotransferases. [Note: An example of the synthesis of a sulfated GAG, chondroitin sulfate, is shown in Figure 14.11.] PAPS is also the sulfur donor in glycosphingolipid synthesis (see p. 210).

A defect in the sulfation of the growing glycosaminoglycan chains results in one of several autosomal recessive disorders, the chondrodystrophies, that affect the proper development and maintenance of the skeletal system.

IV. DEGRADATION OF GLYCOSAMINOGLYCANS

GAGs are degraded in lysosomes, which contain hydrolytic enzymes that are most active at a pH of approximately 5. Therefore, as a group, these enzymes are called acid hydrolases. [Note: The low pH optimum is a protective mechanism that prevents the enzymes from destroying the cell should leakage occur into the cytosol where the pH is neutral.] The half-lives of GAGs vary from minutes to months and are influenced by the type of GAG and its location in the body.

A. Phagocytosis of extracellular glycosaminoglycans

Because GAGs are extracellular or cell-surface compounds, they must first be engulfed by an invagination of the cell membrane (phagocytosis), forming a vesicle inside of which the GAGs are to be degraded. This vesicle then fuses with a lysosome, forming a single digestive vesicle in which the GAGs are efficiently degraded (see p. 150 for a discussion of phagocytosis).

B. Lysosomal degradation of glycosaminoglycans

The lysosomal degradation of GAGs requires a large number of acid hydrolases for complete digestion. First, the polysaccharide chains are cleaved by endoglycosidases, producing oligosaccharides. Further degradation of the oligosaccharides occurs sequentially from the nonreducing end of each chain (see p. 127), the last group (sulfate or sugar) added during synthesis being the first group removed (by sulfatases or exoglycosidases). Examples of some of these enzymes and the bonds they hydrolyze are shown in Figure 14.12. [Note: Endo- and exoglycosidases are also involved in the lysosomal degradation of glycoproteins (see p. 170) and glycolipids (see p. 210). Deficiencies in these enzymes result in the accumulation of partially degraded carbohydrates, resulting in tissue damage.]

Multiple sulfatase deficiency is a rare lysosomal storage disease in which all sulfatases are nonfunctional due to a defect in the formation of formylglycine, an amino acid derivative required at the active site for enzymic activity to occur.

V. MUCOPOLYSACCHARIDOSES

The mucopolysaccharidoses are hereditary diseases (1:25,000 live births) caused by a deficiency of any one of the lysosomal hydrolases normally involved in the degradation of heparan sulfate and/or dermatan sulfate (see Figure 14.12). They are progressive disorders characterized by lysosomal accumulation of GAGs in various tissues, causing a range of symptoms, such as skeletal and extracellular matrix deformities, and intellectual disability. All are autosomal recessive disorders except Hunter syndrome, which is X linked. Children who are homozygous for any one of these diseases are apparently normal at birth and then gradually deteriorate. In severe cases, death occurs in childhood. There currently is no cure. Incomplete lysosomal degradation of GAGs results in the presence of oligosaccharides in the urine. These fragments can be used to diagnose the specific mucopolysaccharidosis by identifying the structure present on the nonreducing end of the oligosaccharide, because that residue would have been the substrate for the missing enzyme. Diagnosis is confirmed by measuring the patient's cellular level of the lysosomal hydrolases. Bone marrow and cord blood transplants, in which transplanted macrophages produce the enzymes that degrade GAGs, have been used to treat Hurler and Hunter syndromes, with limited success. Enzyme replacement therapy is available for both syndromes but does not prevent neurologic damage.

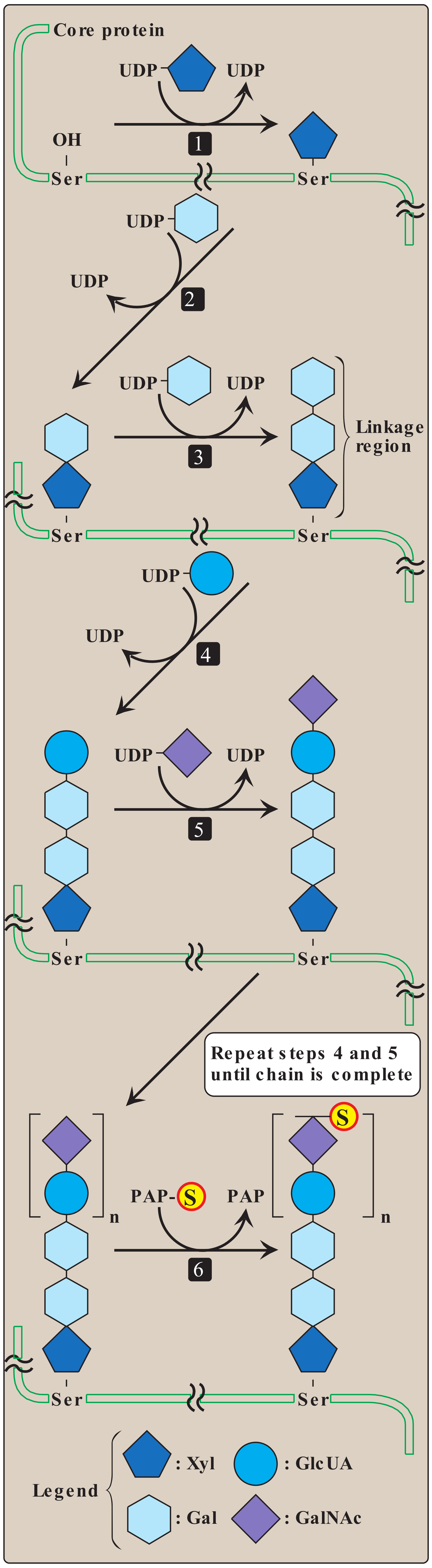
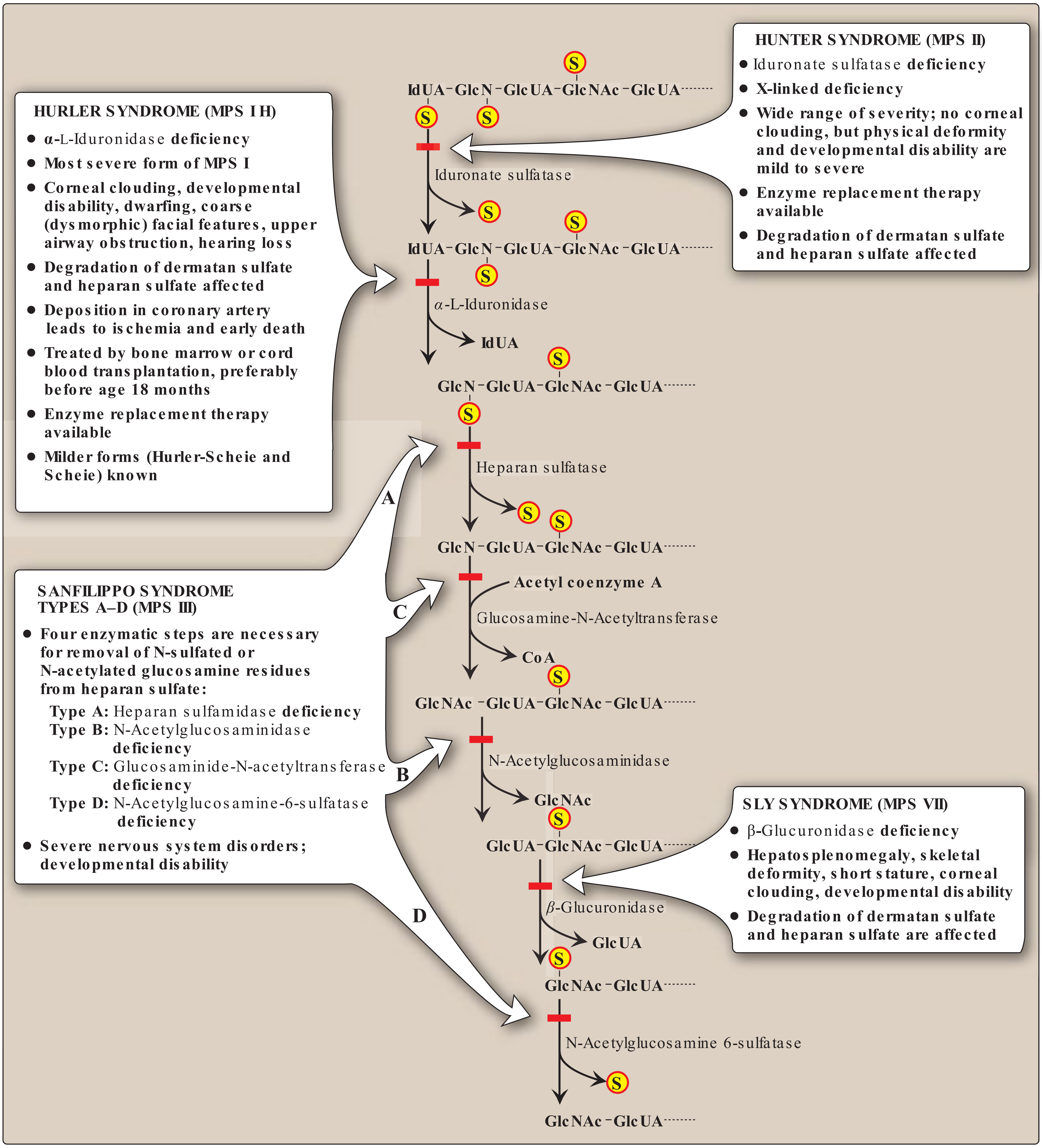


Figure 14.11
Synthesis of chondroitin sulfate.



S
IdUA-GlcN-GlcUA-GlcNAc-GlcUA
S S
Iduronate sulfatase
S
S
IdUA-GlcN-GlcUA-GlcNAc-GlcUA
S
α-L-Iduronidase
IdUA
S
GlcN-GlcUA-GlcNAc-GlcUA
S
Heparan sulfatase
S S
GlcN-GlcUA-GlcNAc-GlcUA
Acetyl coenzyme A
Glucosamine-N-Acetyltransferase
CoA
S
GlcNAc-GlcUA-GlcNAc-GlcUA
N-Acetylglucosaminidase
GlcNAc
S
GlcUA-GlcNAc-GlcUA
β-Glucuronidase
GlcUA
S
GlcNAc-GlcUA
N-Acetylglucosamine 6-sulfatase
S
GlcNAc-GlcUA
A
B
C
D

HURLER SYNDROME (MPS I H)

- α-L-Iduronidase **deficiency**
- **Most severe form of MPS I**
- **Corneal clouding, developmental disability, dwarfing, coarse (dysmorphic) facial features, upper airway obstruction, hearing loss**
- **Degradation of dermatan sulfate and heparan sulfate affected**
- **Deposition in coronary artery leads to ischemia and early death**
- **Treated by bone marrow or cord blood transplantation, preferably before age 18 months**
- **Enzyme replacement therapy available**
- **Milder forms (Hurler-Scheie and Scheie) known**

HUNTER SYNDROME (MPS II)

- Iduronate sulfatase **deficiency**
- **X-linked deficiency**
- **Wide range of severity; no corneal clouding, but physical deformity and developmental disability are mild to severe**
- **Enzyme replacement therapy available**
- **Degradation of dermatan sulfate and heparan sulfate affected**

SANFILIPPO SYNDROME TYPES A–D (MPS III)

- **Four enzymatic steps are necessary for removal of N-sulfated or N-acetylated glucosamine residues from heparan sulfate:**
  - **Type A:** Heparan sulfamidase **deficiency**
  - **Type B:** N-Acetylglucosaminidase **deficiency**
  - **Type C:** Glucosaminide-N-acetyltransferase **deficiency**
  - **Type D:** N-Acetylglucosamine-6-sulfatase **deficiency**
- **Severe nervous system disorders; developmental disability**

SLY SYNDROME (MPS VII)

- β-Glucuronidase **deficiency**
- **Hepatosplenomegaly, skeletal deformity, short stature, corneal clouding, developmental disability**
- **Degradation of dermatan sulfate and heparan sulfate are affected**

Figure 14.12
Degradation of the glycosaminoglycan heparan sulfate by lysosomal enzymes, indicating sites of enzyme deficiencies in some representative mucopolysaccharidoses (MPSs). [Note: Deficiencies in the degradation of keratan sulfate result in Morquio syndrome, A and B. Deficiencies in the degradation of dermatan sulfate result in Maroteaux-Lamy syndrome.] GlcUA = glucuronic acid; IdUA = iduronic acid; GalNAc = N-acetylgalactosamine; GlcNAc = N-acetylglucosamine; GlcN = glucosamine; S = sulfate.

VI. OVERVIEW OF GLYCOPROTEINS

Glycoproteins are proteins to which oligosaccharides are covalently attached. They differ from the proteoglycans in that the length of the carbohydrate chain in glycoproteins is relatively short (usually two to ten sugar residues in length, although they can be longer), whereas it can be very long in the GAGs of proteoglycans (see p. 157). In addition, whereas GAGs have repeating disaccharide units, the carbohydrates of glycoproteins do not have serial repeats. The glycoprotein carbohydrate chains are often branched instead of linear and may or may not be negatively charged. Glycoproteins contain highly variable amounts of carbohydrate but typically less than that in GAGS. For example, immunoglobulin IgG contains less than 4% of its mass as carbohydrate, whereas human gastric glycoprotein (mucin) contains more than 80% carbohydrate. Membrane-bound glycoproteins participate in a broad range of cellular phenomena, including cell-surface recognition (by other cells, hormones, and viruses), cell-surface antigenicity (such as the blood group antigens), and as components of the ECM and of the mucins of the gastrointestinal and urogenital tracts, where they act as protective biologic lubricants. In addition, almost all of the globular proteins present in human plasma are glycoproteins, although albumin is an exception. (See Figure 14.13 for a summary of some of the functions of glycoproteins.) [Note: Glycosylation is the most common posttranslational modification of proteins.]

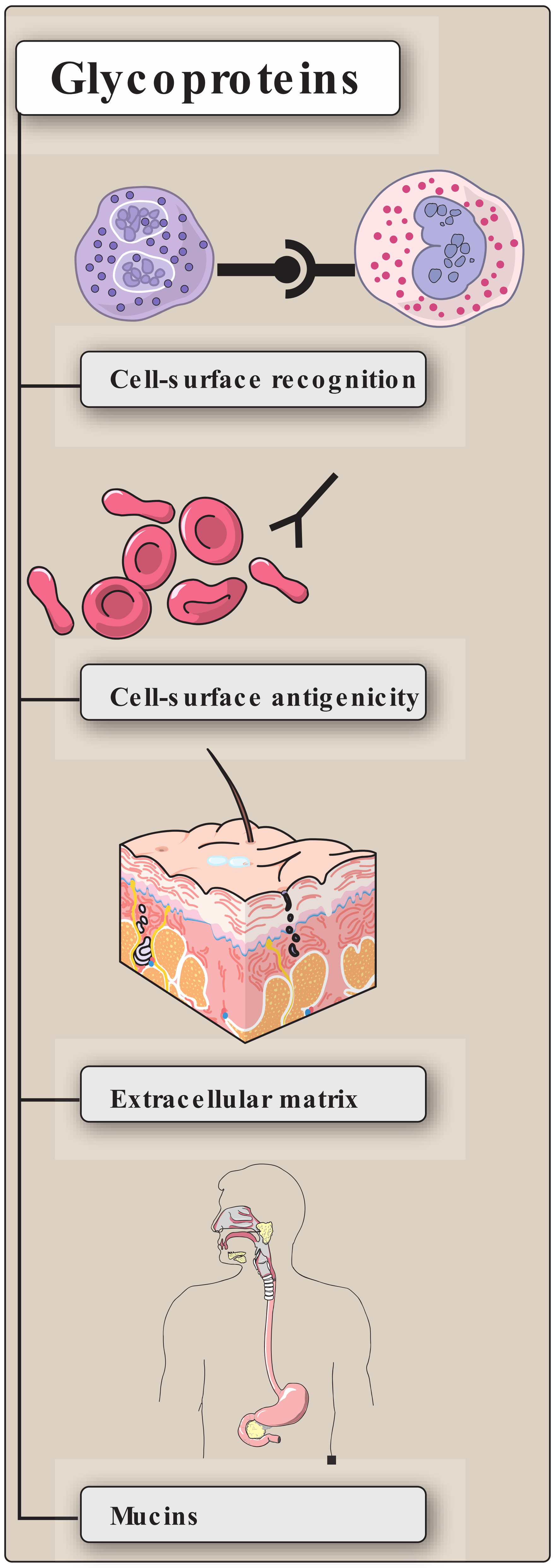


Figure 14.13
Functions of glycoproteins.

VII. OLIGOSACCHARIDE STRUCTURE

The oligosaccharide components of glycoproteins are generally branched heteropolymers composed primarily of D-hexoses, with the addition in some cases of neuraminic acid (a nonose) and of L-fucose, a 6-deoxyhexose.

A. Structure of the linkage between carbohydrate and protein

The oligosaccharide may be attached to the protein through an N- or an O-glycosidic link (see p. 86). In the former case, the sugar chain is attached to the amide group of an asparagine side chain and, in the latter case, to the hydroxyl group of either a serine or threonine side chain. [Note: In the case of collagen, there is an O-glycosidic linkage between galactose or glucose and the hydroxyl group of hydroxylysine (see p. 47).]

B. N- and O-linked oligosaccharides

A glycoprotein may contain only one type of glycosidic linkage (N- or O-linked) or may have both types within the same molecule.

1. **O-Linked oligosaccharides:** The O-linked oligosaccharides may have one or more of a wide variety of sugars arranged in either a linear or a branched pattern. Many O-linked oligosaccharides are found in extracellular glycoproteins or as membrane glycoprotein components. For example, O-linked oligosaccharides on the surface of red blood cells help provide the ABO blood group determinants.

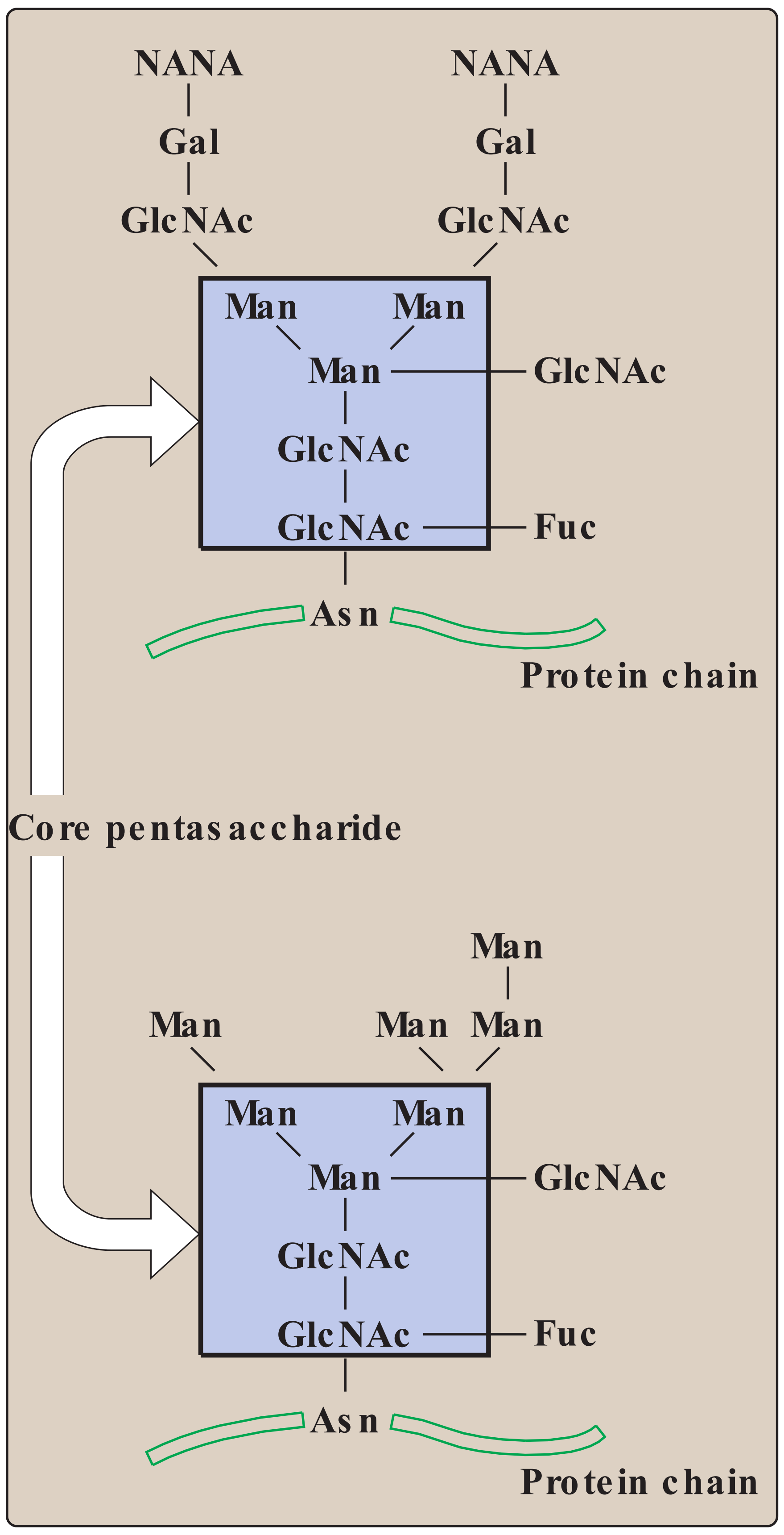


Figure 14.14
Complex (top) and high-mannose (bottom) N-linked oligosaccharides. [Note: Members of each class contain the same pentasaccharide core.] NANA = N-acetylneuraminic acid; Gal = galactose; GlcNAc = N-acetylglucosamine; Man = mannose; Fuc = fucose; Asn = asparagine.

2. **N-linked oligosaccharides:** The N-linked oligosaccharides fall into two broad classes: complex oligosaccharides and high-mannose oligosaccharides. Both contain the same pentasaccharide core shown in Figure 14.14, but the complex oligosaccharides contain a diverse group of additional sugars, for example, GlcNAc, GalNAc, L-fucose, and NANA, whereas the high-mannose oligosaccharides contain primarily mannose.

VIII. SYNTHESIS OF GLYCOPROTEINS

Proteins destined to function in the cytoplasm are synthesized on free cytosolic ribosomes. However, proteins, including glycoproteins, that are destined for cellular membranes, lysosomes, or to be exported from the cell, are synthesized on ribosomes attached to the RER. These proteins contain specific signal sequences that act as molecular "address labels," targeting the proteins to their proper destinations. An N-terminal hydrophobic sequence initially directs these proteins to the RER, allowing the growing polypeptide to be extruded into the lumen. The proteins are then transported via secretory vesicles to the Golgi complex, which acts as a sorting center (Figure 14.15). In the Golgi, those glycoproteins that are to be secreted from the cell (or are targeted for lysosomes) are packaged into vesicles that fuse with the cell (or lysosomal) membrane and release their contents. Those that are destined to become components of the cell membrane are integrated into the Golgi membrane, which buds off, forming vesicles that add their membrane-bound glycoproteins to the cell membrane. [Note: The membrane glycoproteins are, thus, oriented with the carbohydrate portion on the outside of the cell (see Figure 14.15).]

A. Carbohydrate components of glycoproteins

The precursors of the carbohydrate components of glycoproteins are nucleotide sugars, which include UDP-glucose, UDP-galactose, UDP-GlcNAc, and UDP-GalNAc. In addition, guanosine diphosphate (GDP)-mannose, GDP-L-fucose (which is synthesized from GDP-mannose), and CMP-NANA may donate sugars to the growing chain. [Note: When the acidic NANA is present, the oligosaccharide has a negative charge at physiologic pH.] The oligosaccharides are covalently attached to the R groups of specific amino acids in the protein, where the three-dimensional structure of the protein determines whether or not a specific amino acid is glycosylated.

B. Synthesis of O-linked glycosides

The synthesis of the O-linked glycosides is very similar to that of the GAGs (see p. 158). First, the protein to which the oligosaccharides are to be attached is synthesized on the RER and extruded into its lumen. Glycosylation begins with the transfer of GalNAc (from UDP-GalNAc) onto the R-group of a specific serine or threonine.

1. **Role of glycosyltransferases:** The glycosyltransferases responsible for the stepwise synthesis of the oligosaccharides are bound to the membranes of the Golgi apparatus. They act in a specific order, without using a template as is required for DNA, RNA, and protein synthesis (see Unit VI) but, rather by recognizing the

actual structure of the growing oligosaccharide as the appropriate substrate.

C. Synthesis of the N-linked glycosides

The synthesis of N-linked glycosides occurs in the lumen of the RER and requires the participation of the phosphorylated form of dolichol (dolichol pyrophosphate), a lipid of the ER membrane (Figure 14.16). The initial product is processed in the ER and Golgi.

1. **Synthesis of dolichol-linked oligosaccharide:** First, as with the O-linked glycosides, the protein is synthesized on the RER and enters its lumen. However, the protein does not become glycosylated with individual sugars. Instead, a lipid-linked oligosaccharide is first constructed.This consists of dolichol (an ER membrane lipid 80–100 carbons long) attached through a pyrophosphate linkage to an oligosaccharide containing N-GlcNAc, mannose, and glucose. The sugars to be added sequentially to the dolichol by the membrane-bound glycosyltransferases are first N-GlcNAc, followed by mannose and glucose (see Figure 14.16). The entire 14-sugar oligosaccharide is then transferred from the dolichol to the amide nitrogen (N) of an asparagine in the protein to be glycosylated by a protein-oligosaccharide transferase present in the ER. [Note: Tunicamycin inhibits N-linked glycosylation.]

Congenital disorders of glycosylation (CDGs) are syndromes caused primarily by defects in the N-linked glycosylation of proteins, either oligosaccharide assembly (Type I) or processing (Type II).

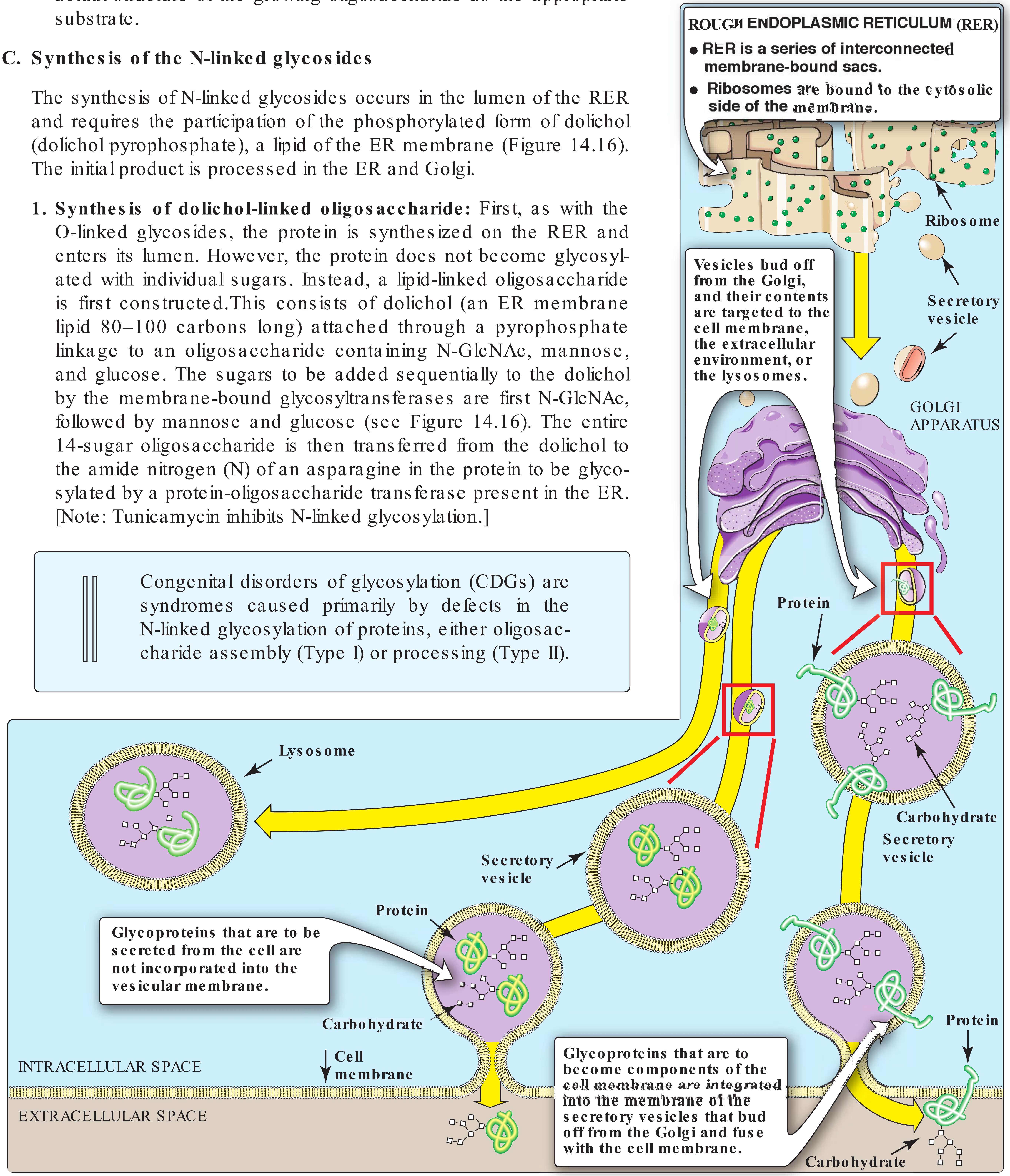
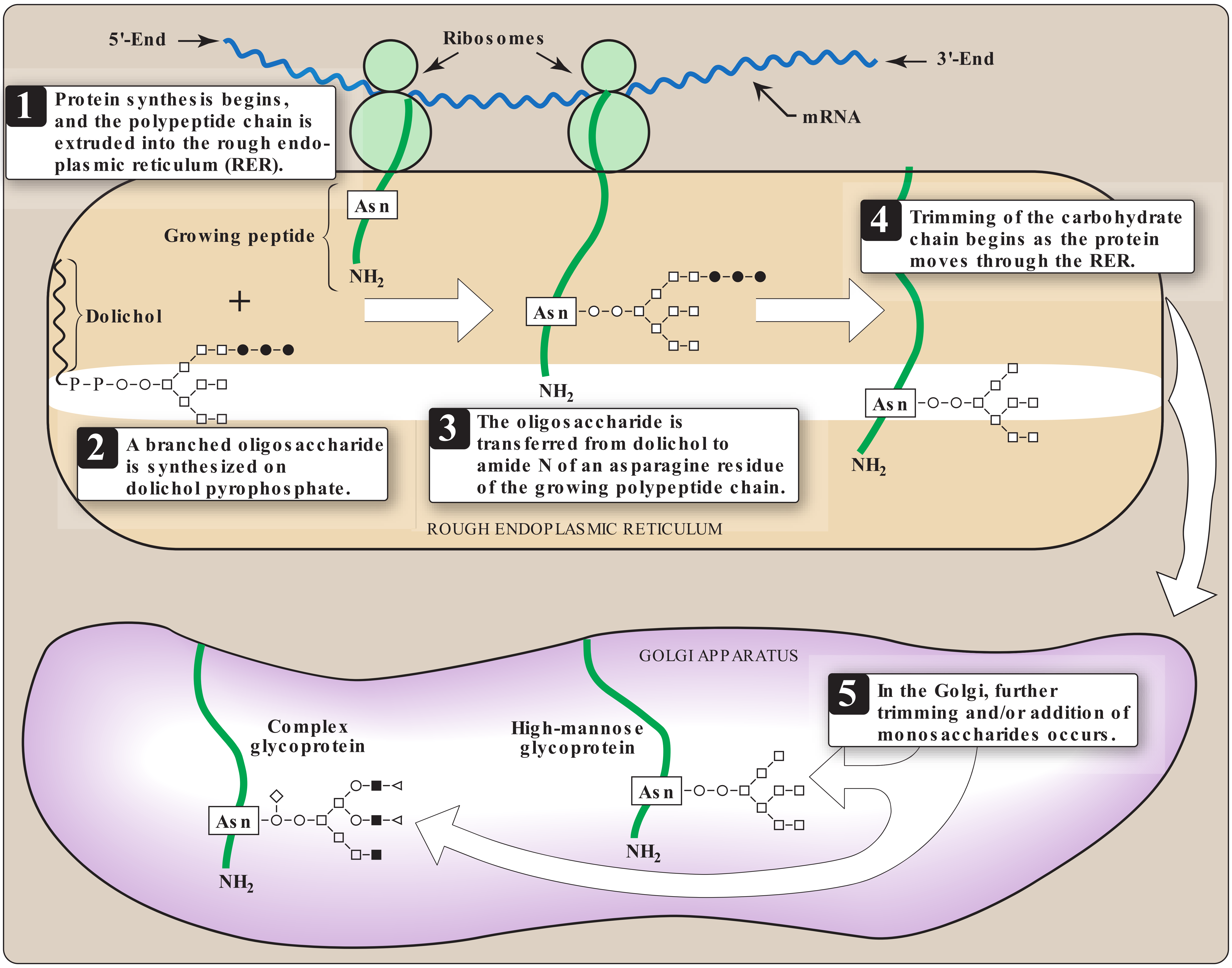

ROUGH ENDOPLASMIC RETICULUM (RER)
● RER is a series of interconnected membrane-bound sacs.
● Ribosomes are bound to the cytosolic side of the membrane.
Ribosome
Secretory vesicle
Vesicles bud off from the Golgi, and their contents are targeted to the cell membrane, the extracellular environment, or the lysosomes.
GOLGI APPARATUS
Protein
Carbohydrate
Secretory vesicle
Lysosome
Secretory vesicle
Protein
Glycoproteins that are to be secreted from the cell are not incorporated into the vesicular membrane.
Carbohydrate
Cell membrane
INTRACELLULAR SPACE
EXTRACELLULAR SPACE
Glycoproteins that are to become components of the cell membrane are integrated into the membrane of the secretory vesicles that bud off from the Golgi and fuse with the cell membrane.
Protein
Carbohydrate

Figure 14.15
Transport of glycoproteins through the Golgi apparatus and their subsequent release or incorporation into a lysosome or the cell membrane.



1 Protein synthesis begins, and the polypeptide chain is extruded into the rough endoplasmic reticulum (RER).

2 A branched oligosaccharide is synthesized on dolichol pyrophosphate.

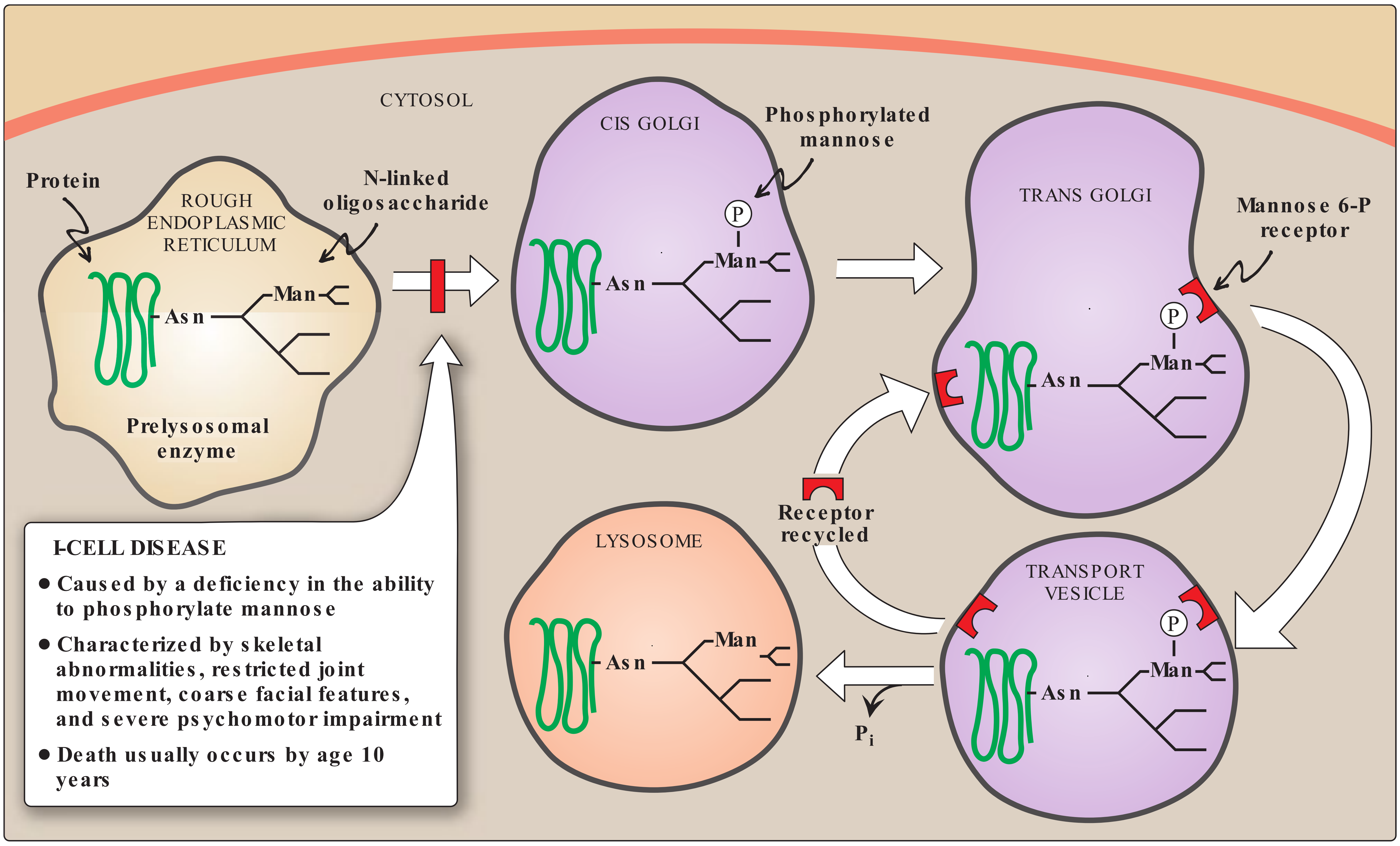
3 The oligosaccharide is transferred from dolichol to amide N of an asparagine residue of the growing polypeptide chain.

4 Trimming of the carbohydrate chain begins as the protein moves through the RER.

5 In the Golgi, further trimming and/or addition of monosaccharides occurs.

Figure 14.16
Synthesis of N-linked glycoproteins. ○ = N-acetylglucosamine; □ = mannose; ● = glucose; ■ = N-acetylgalactosamine; ◇ or ◁ = terminal group (fucose or N-acetylneuraminic acid); mRNA = messenger RNA; Asn = asparagine.

2. **Final processing of N-linked oligosaccharides:** After incorporation into the protein, the N-linked oligosaccharide is processed by the removal of specific mannosyl and glucosyl residues as the glycoprotein moves through the RER. Finally, the oligosaccharide chains are completed in the Golgi by addition of a variety of sugars (for example, N-GlcNAc, N-GalNAc, and additional mannoses, and then fucose or NANA as terminal groups) to produce a complex glycoprotein. Alternatively, they are not processed further, leaving branched, mannose-containing chains in a high-mannose glycoprotein (see Figure 14.16). The ultimate fate of N-linked glycoproteins is the same as that of the O-glycoproteins linked (for example, they can be released by the cell or become part of a cell membrane). In addition, N-linked glycoproteins can be targeted to the lysosomes. [Note: Nonenzymatic glycosylation of proteins is known as glycation (see p. 345).]


CYTOSOL
Protein
ROUGH ENDOPLASMIC RETICULUM
N-linked oligosaccharide
Asn
Man
Prelysosomal enzyme
CIS GOLGI
Phosphorylated mannose
P
Asn
Man
TRANS GOLGI
Mannose 6-P receptor
P
Asn
Man
Receptor recycled
LYSOSOME
Asn
Man
P_i
TRANSPORT VESICLE
P
Asn
Man

I-CELL DISEASE

- **Caused by a deficiency in the ability to phosphorylate mannose**
- **Characterized by skeletal abnormalities, restricted joint movement, coarse facial features, and severe psychomotor impairment**
- **Death usually occurs by age 10 years**

Figure 14.17
Mechanism for transport of N-linked glycoproteins to the lysosomes. Asn = asparagine; Man = mannose; P = phosphate; P_i = inorganic phosphate.

3. **Enzymes destined for lysosomes:** N-linked glycoproteins being processed through the Golgi can be phosphorylated on carbon 6 of one or more specific mannosyl residues. Mannose 6-phosphate receptors, located in the Golgi apparatus, bind the mannose 6-phosphate residues of these targeted enzymes, which are then packaged into vesicles and sent to the lysosomes. I-cell disease is a rare lysosomal storage disease in which the acid hydrolases normally found in lysosomes are absent, resulting in an accumulation of substrates normally degraded by these enzymes. [Note: I-cell disease is so-named because of the large inclusion bodies seen in cells of patients with this disease.] In addition, high amounts of lysosomal enzymes are found in the patient's plasma and urine, indicating that the targeting process to lysosomes (rather than the synthetic pathway of these enzymes) is deficient. Individuals with I-cell disease are lacking the phosphotransferase needed to phosphorylate the mannose residues of potential lysosomal enzymes, causing the enzymes to be secreted (by default), rather than being targeted to lysosomal vesicles (Figure 14.17). I-cell disease is characterized by skeletal abnormalities, restricted joint movement, coarse (dysmorphic) facial features, and severe psychomotor impairment. [Note: Because I-cell disease has features in common with the mucopolysaccharidoses and sphingolipidoses (see p. 211), it is termed a mucolipidosis.] Currently, there is no cure, and death from cardiopulmonary complications usually occurs by age 10 years.

IX. LYSOSOMAL DEGRADATION OF GLYCOPROTEINS

Degradation of glycoproteins is similar to that of the GAGs (see p. 162). The lysosomal acid hydrolases are each generally specific for the removal of one component of the glycoprotein. They are primarily exoenzymes that remove their respective groups in the reverse order of their incorporation ("last on, first off"). If any one degradative enzyme is missing, degradation by the other exoenzymes cannot continue. A group of very rare, autosomal recessive diseases called the glycoprotein storage diseases (oligosaccharidoses), caused by a deficiency of any one of the degradative enzymes, results in accumulation of partially degraded structures in the lysosomes. For example, α-mannosidosis type 1 is a progressive, fatal deficiency of the enzyme, α-mannosidase. Presentation is similar to Hurler syndrome, but immune deficiency is also seen. Mannose-rich oligosaccharide fragments appear in the urine. Diagnosis is by enzyme assay.

X. CHAPTER SUMMARY

Glycosaminoglycans (**GAGs**) are **long**, **negatively charged**, **unbranched**, **heteropolysaccharide chains** generally composed of a **repeating disaccharide unit** [acidic sugar–amino sugar]$_n$ (Figure 14.18). The **amino sugar** is either **D-glucosamine** or **D-galactosamine** in which the amino group is usually acetylated, thus eliminating its positive charge. The amino sugar may also be sulfated on carbon 4 or 6 or on a nonacetylated nitrogen. The **acidic sugar** is either **D-glucuronic acid** or its C-5 epimer **L-iduronic acid**. GAGs **bind large amounts of water**, thereby producing the gel-like matrix that forms the basis of the body's **ground substance.** The viscous, lubricating properties of **mucous secretions** are also caused by the presence of GAGs, which led to the original naming of these compounds as **mucopolysaccharides.** There are **six major types** of GAGs, including **chondroitin 4- and 6-sulfates**, **keratan sulfate**, **dermatan sulfate**, **heparin**, **heparan sulfate**, and **hyaluronic acid**. All of the GAGs, except hyaluronic acid, are found covalently attached to protein, forming **proteoglycan monomers**, which consist of a **core protein** to which the linear GAG chains are covalently attached. The proteoglycan monomers associate with a molecule of **hyaluronic acid** to form **proteoglycan aggregates**. GAGs are synthesized in the **Golgi**. The polysaccharide chains are elongated by the sequential addition of alternating acidic and amino sugars, donated by their **UDP-derivatives**. D-glucuronate may be epimerized to L-iduronate. The last step in synthesis is sulfation of some of the amino sugars. The source of the sulfate is **3′-phosphoadenosyl-5′-phosphosulfate**. The completed proteoglycans are secreted into the extracellular matrix or remain associated with the outer surface of cells. GAGs are **degraded** by **lysosomal** acid hydrolases. They are first broken down to oligosaccharides, which are degraded sequentially from the nonreducing end of each chain. A **deficiency** of any one of the hydrolases results in a **mucopolysaccharidosis**. These are hereditary disorders in which GAGs accumulate in tissues, causing symptoms such as **skeletal** and **extracellular matrix deformities** and **intellectual disability**. Examples of these genetic diseases include **Hunter** and **Hurler syndromes**. **Glycoproteins** are proteins to which **oligosaccharides** are covalently attached. They differ from the proteoglycans in that the length of the glycoprotein's carbohydrate chain is relatively short (usually two to ten sugar residues long, although they can be longer), may be branched, and does not contain serial disaccharide units. **Membrane-bound** glycoproteins participate in a broad range of cellular phenomena, including **cell-surface recognition** (by other cells, hormones, and viruses), **cell-surface antigenicity** (such as the blood group antigens), and as components of the **extracellular matrix** and of the **mucins** of the gastrointestinal and urogenital tracts, where they act as protective biologic lubricants. In addition, almost all of the globular proteins present in human plasma are glycoproteins. Glycoproteins are **synthesized** in the **rough endoplasmic reticulum** (**RER**) and the **Golgi**. The precursors of the carbohydrate components of glycoproteins are **nucleotide sugars**. **O-linked glycoproteins** are synthesized in the Golgi by the sequential transfer of sugars from their nucleotide carriers to the hydroxyl group of a serine or threonine residue in the protein. **N-linked glycoproteins** contain varying amounts of **mannose**. They are synthesized by the transfer of a preformed oligosaccharide from its ER membrane lipid carrier, **dolichol pyrophosphate**, to the amide N of an asparagine residue in the protein. A deficiency in the phosphorylation of mannose residues in N-linked glycoprotein enzymes destined for the lysosomes results in **I-cell disease**. Glycoproteins are degraded in lysosomes by acid hydrolases. A deficiency of any one of these enzymes results in a lysosomal **glycoprotein storage disease** (**oligosaccharidosis**), resulting in accumulation of partially degraded structures in the lysosome.

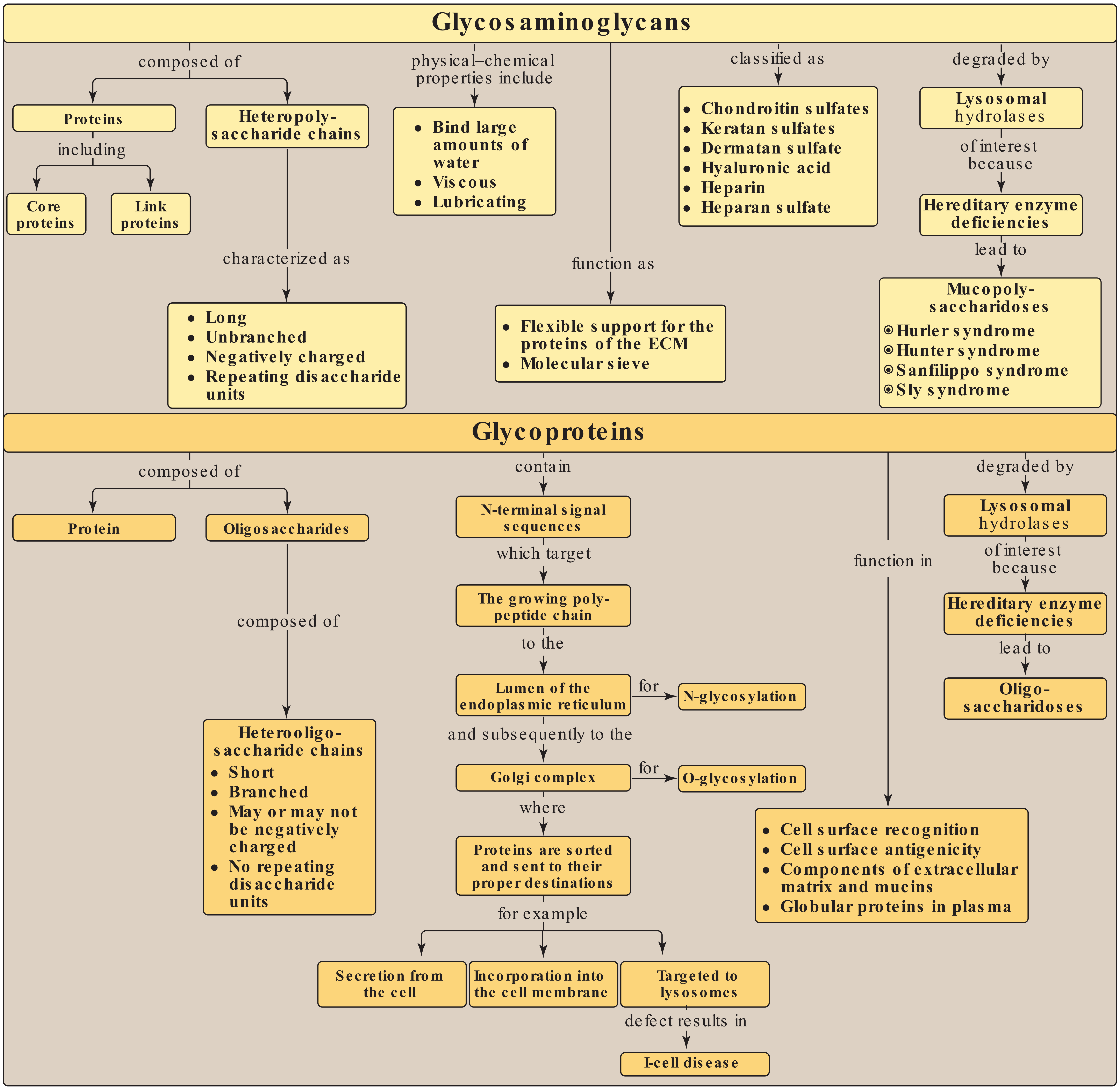

Glycosaminoglycans
composed of
Proteins
including
Core proteins
Link proteins
Heteropoly-saccharide chains
characterized as
• Long
• Unbranched
• Negatively charged
• Repeating disaccharide units
physical–chemical properties include
• Bind large amounts of water
• Viscous
• Lubricating
function as
• Flexible support for the proteins of the ECM
• Molecular sieve
classified as
• Chondroitin sulfates
• Keratan sulfates
• Dermatan sulfate
• Hyaluronic acid
• Heparin
• Heparan sulfate
degraded by
Lysosomal hydrolases
of interest because
Hereditary enzyme deficiencies
lead to
Mucopoly-saccharidoses
⦿ Hurler syndrome
⦿ Hunter syndrome
⦿ Sanfilippo syndrome
⦿ Sly syndrome
Glycoproteins
composed of
Protein
Oligosaccharides
composed of
Heterooligo-saccharide chains
• Short
• Branched
• May or may not be negatively charged
• No repeating disaccharide units
contain
N-terminal signal sequences
which target
The growing poly-peptide chain
to the
Lumen of the endoplasmic reticulum
for
N-glycosylation
and subsequently to the
Golgi complex
for
O-glycosylation
where
Proteins are sorted and sent to their proper destinations
for example
Secretion from the cell
Incorporation into the cell membrane
Targeted to lysosomes
defect results in
I-cell disease
function in
• Cell surface recognition
• Cell surface antigenicity
• Components of extracellular matrix and mucins
• Globular proteins in plasma
degraded by
Lysosomal hydrolases
of interest because
Hereditary enzyme deficiencies
lead to
Oligo-saccharidoses

Figure 14.18
Key concept map for glycosaminoglycans and glycoproteins. ECM = extracellular matrix.

UNIT III:
Lipid Metabolism

Dietary Lipid Metabolism

15

I. OVERVIEW

Lipids are a heterogeneous group of water-insoluble (hydrophobic) organic molecules (Figure 15.1). Because of their insolubility in aqueous solutions, body lipids are generally found compartmentalized, as in the case of membrane-associated lipids or droplets of triacylglycerol in adipocytes, or transported in plasma in association with protein, as in lipoprotein particles (see p. 227), or on albumin. Lipids are a major source of energy for the body, and they also provide the hydrophobic barrier that permits partitioning of the aqueous contents of cells and subcellular structures. Lipids serve additional functions in the body (for example, some fat-soluble vitamins have regulatory or coenzyme functions, and the prostaglandins and steroid hormones play major roles in the control of the body's homeostasis). Not surprisingly, deficiencies or imbalances of lipid metabolism can lead to some of the major clinical problems encountered by physicians, such as atherosclerosis, diabetes, and obesity.

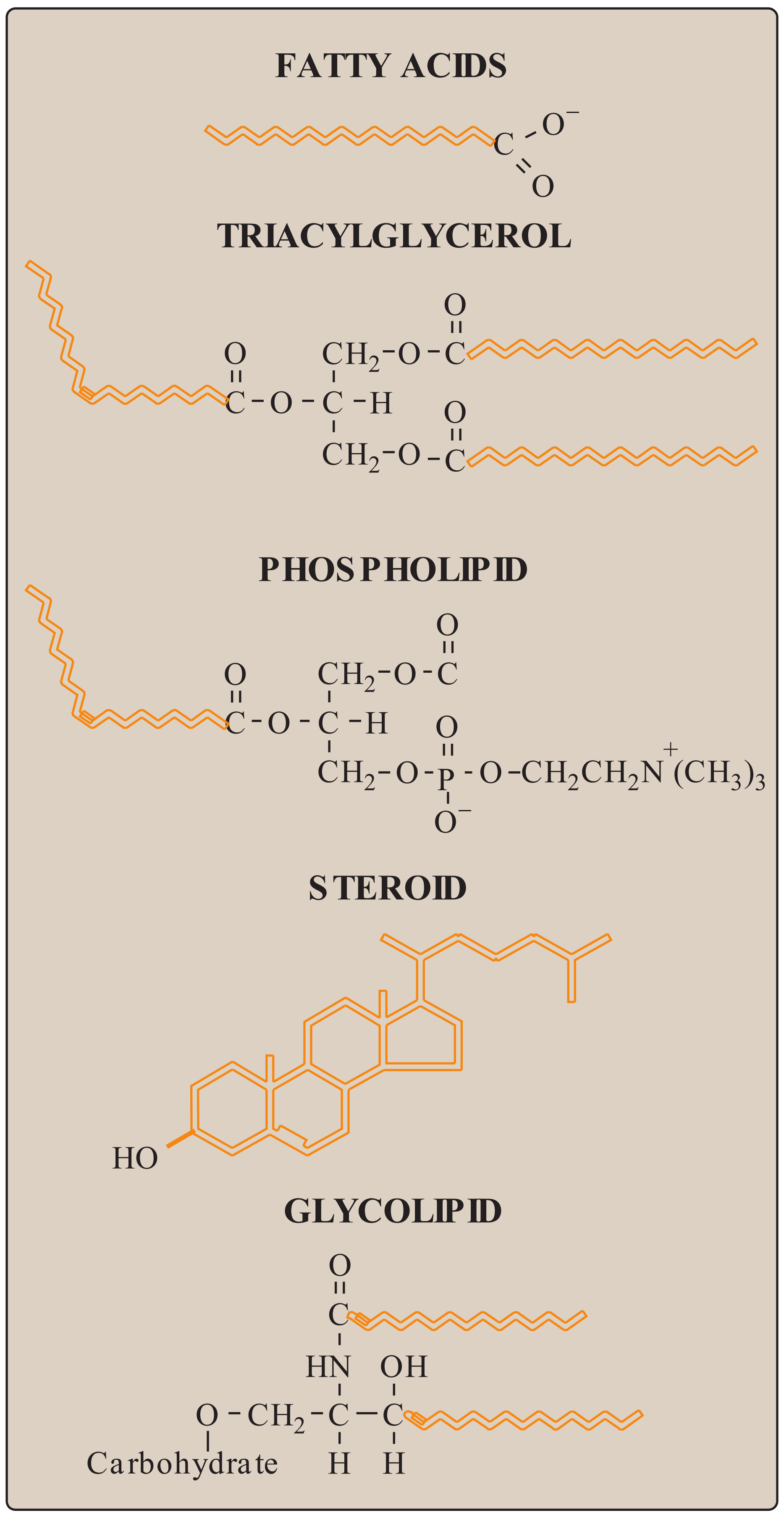


Figure 15.1
Structures of some common classes of lipids. Hydrophobic portions of the molecules are shown in orange.

II. DIGESTION, ABSORPTION, SECRETION, AND UTILIZATION OF DIETARY LIPIDS

The average daily intake of lipids by U.S. adults is about 81 g, of which more than 90% is normally triacylglycerol ([TAG], formerly called triglyceride). The remainder of the dietary lipids consists primarily of cholesterol, cholesteryl esters, phospholipids, and unesterified ("free") fatty acids. The digestion of dietary lipids is summarized in Figure 15.2.

A. Processing of dietary lipid in the stomach

The digestion of lipids begins in the stomach, catalyzed by a lipase (lingual lipase) that originates from glands at the back of the tongue. TAG molecules, particularly those containing fatty acids of short- or medium-chain length (fewer than 12 carbons such as are found in milk fat), are the primary target of this enzyme. These same TAGs are also degraded by a separate gastric lipase, secreted by the gastric mucosa. Both enzymes are relatively

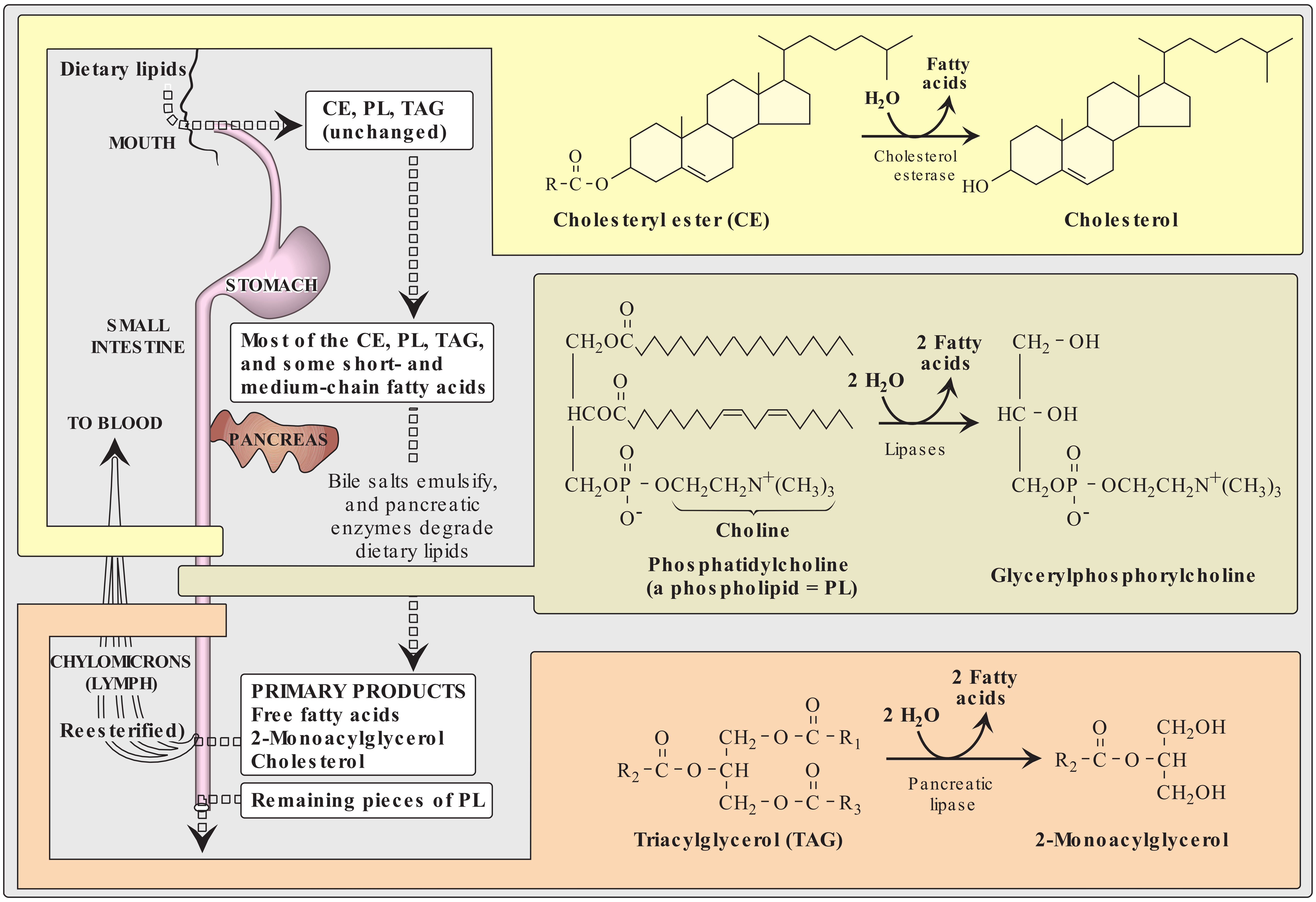

Dietary lipids
MOUTH
CE, PL, TAG (unchanged)
STOMACH
SMALL INTESTINE
Most of the CE, PL, TAG, and some short- and medium-chain fatty acids
PANCREAS
TO BLOOD
Bile salts emulsify, and pancreatic enzymes degrade dietary lipids
CHYLOMICRONS (LYMPH)
Reesterified)
PRIMARY PRODUCTS
Free fatty acids
2-Monoacylglycerol
Cholesterol
Remaining pieces of PL
R-C-O
HO
H2O
Fatty acids
Cholesterol esterase
Cholesteryl ester (CE)
Cholesterol
CH2OC
HCOC
CH2OP - OCH2CH2N+(CH3)3
O-
Choline
2 H2O
2 Fatty acids
Lipases
CH2 - OH
HC - OH
Phosphatidylcholine (a phospholipid = PL)
Glycerylphosphorylcholine
CH2 - O - C - R1
R2 - C - O - CH
CH2 - O - C - R3
Pancreatic lipase
R2 - C - O - CH
CH2OH
Triacylglycerol (TAG)
2-Monoacylglycerol

Figure 15.2
Overview of lipid digestion.

acid stable, with pH optimums of pH 4 to pH 6. These "acid lipases" play a particularly important role in lipid digestion in neonates, for whom milk fat is the primary source of calories. They also become important digestive enzymes in individuals with pancreatic insufficiency such as those with cystic fibrosis (CF). Lingual and gastric lipases aid these patients in degrading TAG molecules (especially those with short- to medium-chain length fatty acids) despite a near or complete absence of pancreatic lipase (see below).

1. **Cystic fibrosis:** CF is the most common lethal genetic disease in Caucasians of Northern European ancestry and has a prevalence of about 1:3,300 births in the United States. CF is an autosomal recessive disorder caused by mutations to the gene for the CF transmembrane conductance regulator (CFTR) protein that functions as a chloride channel on epithelium in the pancreas, lungs, testes, and sweat gands. Defective CFTR results in decreased secretion of chloride and increased uptake of sodium and water. In the pancreas, the depletion of water on the cell surface results in thickened secretions that clog the pancreatic ducts, preventing pancreatic enzymes from reaching the intestine, thereby leading to pancreatic insufficiency. Treatment includes replacement of these enzymes and supplementation with fat-soluble vitamins. [Note: CF also causes chronic lung infections with progressive pulmonary disease and male infertility.]

B. Emulsification of dietary lipid in the small intestine

The critical process of emulsification of dietary lipids occurs in the duodenum. Emulsification increases the surface area of the hydrophobic lipid droplets so that the digestive enzymes, which work at the interface of the droplet and the surrounding aqueous solution, can act effectively. Emulsification is accomplished by two complementary mechanisms, namely, use of the detergent properties of the conjugated bile salts and mechanical mixing due to peristalsis. Bile salts, made in the liver and stored in the gallbladder, are amphipathic derivatives of cholesterol (see p. 224). Conjugated bile salts consist of a hydroxylated sterol ring structure with a side chain to which a molecule of glycine or taurine is covalently attached by an amide linkage (Figure 15.3). These emulsifying agents interact with the dietary lipid particles and the aqueous duodenal contents, thereby stabilizing the particles as they become smaller from peristalsis and preventing them from coalescing. A more complete discussion of bile salt metabolism is given on p. 225.

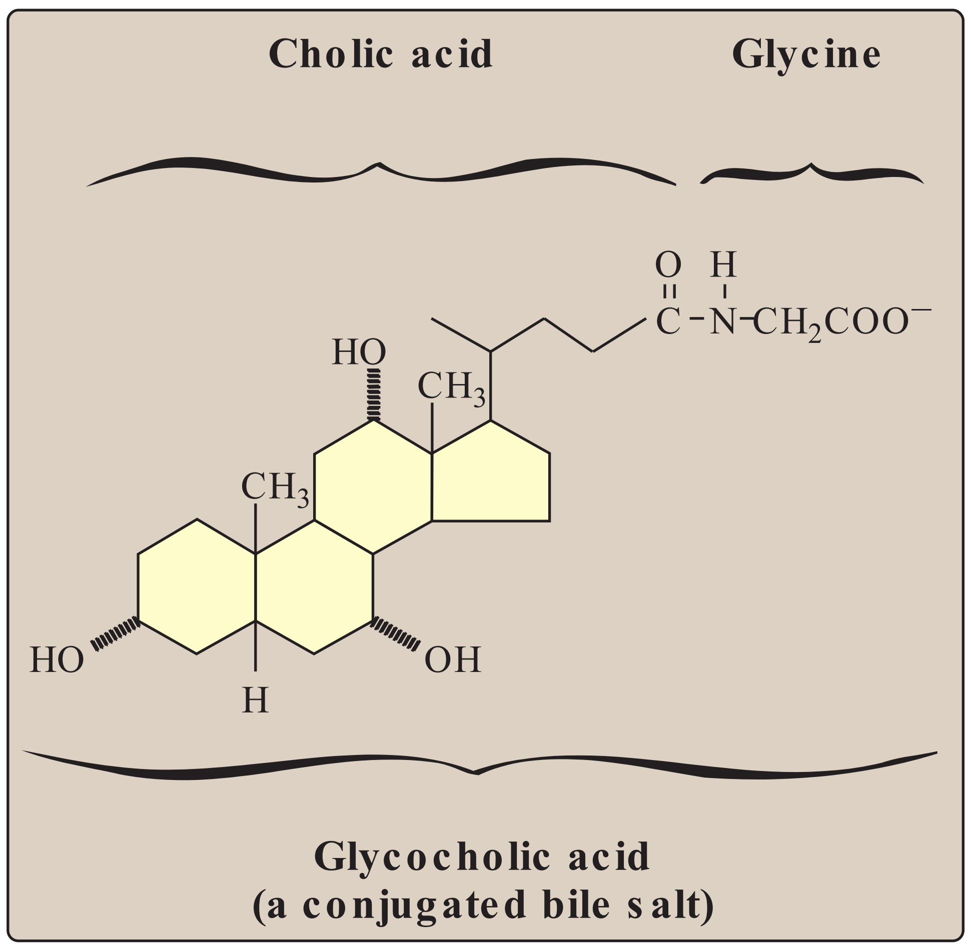


Figure 15.3
Structure of glycocholic acid.

C. Degradation of dietary lipids by pancreatic enzymes

The dietary TAG, cholesteryl esters, and phospholipids are enzymically degraded ("digested") by pancreatic enzymes, whose secretion is hormonally controlled.

1. **Triacylglycerol degradation:** TAG molecules are too large to be taken up efficiently by the mucosal cells of the intestinal villi. They are, therefore, acted upon by an esterase, pancreatic lipase, which preferentially removes the fatty acids at carbons 1 and 3. The primary products of hydrolysis are, thus, a mixture of 2-monoacylglycerol and free fatty acids (see Figure 15.2). [Note: This enzyme is found in high concentrations in pancreatic secretions (2%–3% of the total protein present), and it is highly efficient catalytically, thus insuring that only severe pancreatic deficiency, such as that seen in CF, results in significant malabsorption of fat.] A second protein, colipase, also secreted by the pancreas, binds the lipase at a ratio of 1:1 and anchors it at the lipid–aqueous interface. Colipase restores activity to lipase in the presence of inhibitory substances like bile salts that bind the micelles. [Note: Colipase is secreted as the zymogen, procolipase, which is activated in the intestine by trypsin.] Orlistat, an antiobesity drug, inhibits gastric and pancreatic lipases, thereby decreasing fat absorption, resulting in weight loss.

2. **Cholesteryl ester degradation:** Most dietary cholesterol is present in the free (nonesterified) form, with 10%–15% present in the esterified form. Cholesteryl esters are hydrolyzed by pancreatic cholesteryl ester hydrolase (cholesterol esterase), which produces cholesterol plus free fatty acids (see Figure 15.2). Activity of this enzyme is greatly increased in the presence of bile salts.

3. **Phospholipid degradation:** Pancreatic juice is rich in the proenzyme of phospholipase A_2 that, like procolipase, is activated by trypsin and, like cholesteryl ester hydrolase, requires bile salts for optimum activity. Phospholipase A_2 removes one fatty acid from carbon 2 of a phospholipid, leaving a lysophospholipid. For example, phosphatidylcholine (the predominant phospholipid of

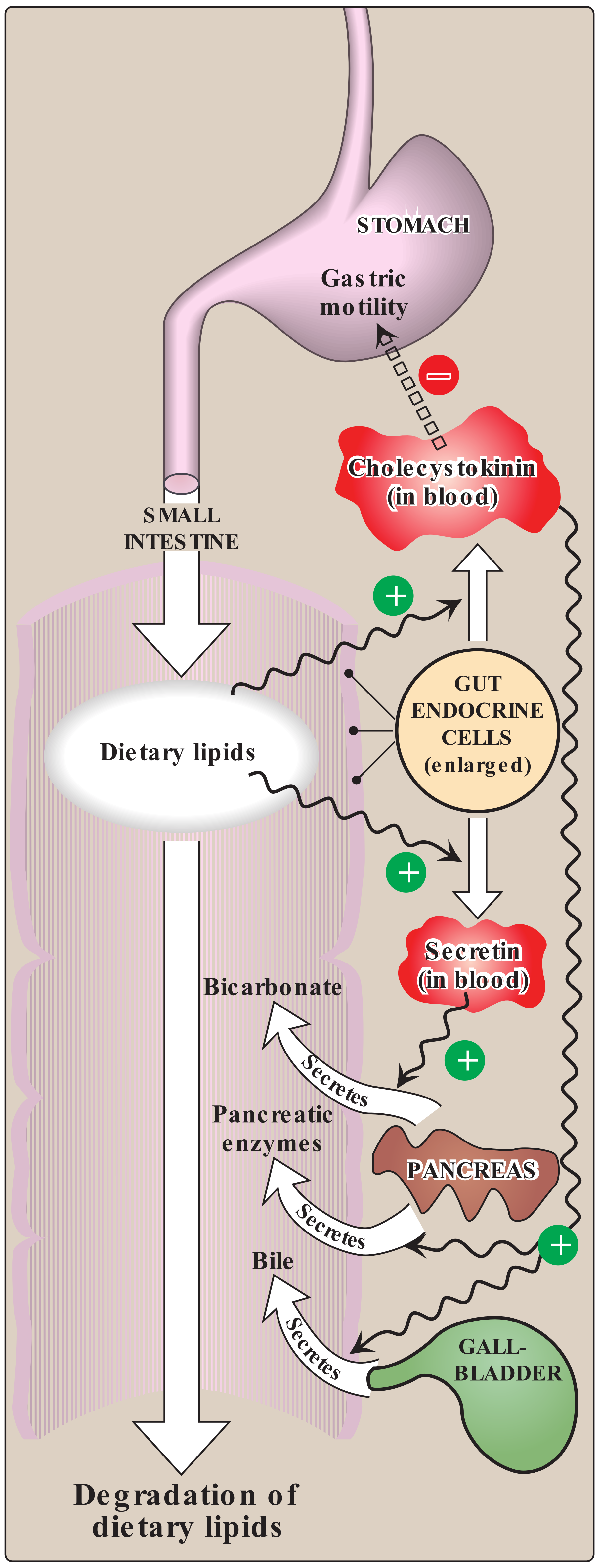


Figure 15.4
Hormonal control of lipid digestion in the small intestine.

digestion) becomes lysophosphatidylcholine. The remaining fatty acid at carbon 1 can be removed by lysophospholipase, leaving a glycerylphosphoryl base (for example, glycerylphosphorylcholine, see Figure 15.2) that may be excreted in the feces, further degraded, or absorbed.

4. **Control of lipid digestion:** Pancreatic secretion of the hydrolytic enzymes that degrade dietary lipids in the small intestine is hormonally controlled (Figure 15.4). Cells in the mucosa of the lower duodenum and jejunum produce a small peptide hormone, cholecystokinin (CCK), in response to the presence of lipids and partially digested proteins entering these regions of the upper small intestine. CCK acts on the gallbladder (causing it to contract and release bile, a mixture of bile salts, phospholipids, and free cholesterol) and on the exocrine cells of the pancreas (causing them to release digestive enzymes). It also decreases gastric motility, resulting in a slower release of gastric contents into the small intestine (see p. 353). Other intestinal cells produce another small peptide hormone, secretin, in response to the low pH of the chyme entering the intestine. Secretin causes the pancreas to release a solution rich in bicarbonate that helps neutralize the pH of the intestinal contents, bringing them to the appropriate pH for digestive activity by pancreatic enzymes.

D. Absorption of lipids by intestinal mucosal cells, or enterocytes

Free fatty acids, free cholesterol, and 2-monoacylglycerol are the primary products of lipid digestion in the jejunum. These, plus bile salts and fat-soluble vitamins (A, D, E, and K), form mixed micelles (that is, disc-shaped clusters of a mixture of amphipathic lipids that coalesce with their hydrophobic groups on the inside and their hydrophilic groups on the outside). Mixed micelles are, therefore, soluble in the aqueous environment of the intestinal lumen (Figure 15.5). These particles approach the primary site of lipid absorption, the brush border membrane of the enterocytes (mucosal cell). This membrane is separated from the liquid contents of the intestinal lumen by an unstirred water layer that mixes poorly with the bulk fluid. The hydrophilic surface of the micelles facilitates the transport of the hydrophobic lipids through the unstirred water layer to the brush border membrane where they are absorbed. Bile salts are absorbed in the terminal ileum, with less than 5% being lost in the feces. [Note: Relative to other dietary lipids, cholesterol is only poorly absorbed by the enterocytes. Drug therapy (for example, with ezetimibe) can further reduce cholesterol absorption in the small intestine.] Short- and medium-chain length fatty acids are water soluble and, thus, do not require the assistance of mixed micelles for absorption by the intestinal mucosa.

E. Resynthesis of triacylglycerols and cholesteryl esters

The mixture of lipids absorbed by the enterocytes migrates to the endoplasmic reticulum where biosynthesis of complex lipids takes place. The long-chain length fatty acids are first converted into their activated form by fatty acyl-coenzyme A (CoA) synthetase (thiokinase) as shown in Figure 15.6. Using the fatty acyl CoA derivatives, the 2-monoacylglycerols absorbed by the enterocytes are converted to TAGs through sequential reacylations by two acyltransferases, acyl CoA:monoacylglycerol acyltransferase and acyl CoA:diacylglycerol acyltransferase. Lysophospholipids are reacylated to form phospholipids by a family of acyltransferases, and cholesterol is esterified with a fatty acid primarily by acyl CoA:cholesterol acyltransferase (see p. 232). [Note: Virtually all long-chain fatty acids entering the enterocytes are used in this fashion to form TAGs, phospholipids, and cholesteryl esters. Short- and medium-chain length fatty acids are not converted to their CoA derivatives and are not reesterified to 2-monoacylglycerol. Instead, they are released into the portal circulation, where they are carried by serum albumin to the liver.]

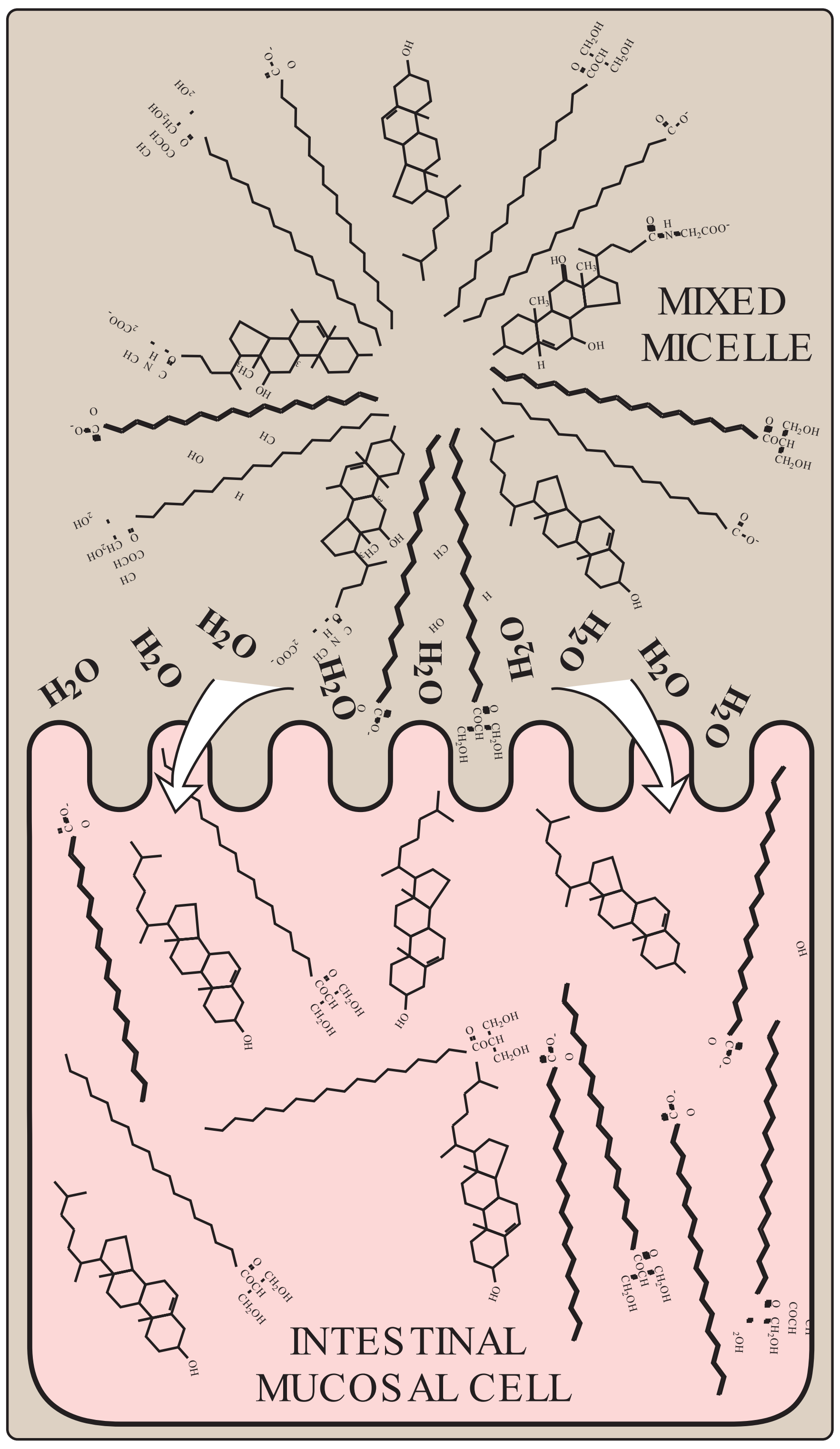

MIXED MICELLE
H_2O
H_2O
H_2O
H_2O
H_2O
H_2O
H_2O
H_2O
INTESTINAL MUCOSAL CELL

Figure 15.5
Absorption of lipids contained in a mixed micelle by an intestinal mucosal cell. [Note: The micelle itself is not taken up.]

F. Lipid malabsorption

Lipid malabsorption, resulting in increased lipid (including the fat-soluble vitamins and essential fatty acids, see p. 182) in the feces, a condition known as steatorrhea, can be caused by disturbances in lipid digestion and/or absorption (Figure 15.7). Such disturbances can result from several conditions, including CF (causing poor digestion) and short bowel syndrome (causing decreased absorption).

> The ability of short- and medium-chain length fatty acids to be taken up by enterocytes without the aid of mixed micelles has made them important in dietary therapy for individuals with malabsorption disorders.

G. Secretion of lipids from enterocytes

The newly resynthesized TAGs and cholesteryl esters are very hydrophobic and aggregate in an aqueous environment. It is, therefore, necessary that they be packaged as particles of lipid droplets surrounded by a thin layer composed of phospholipids, unesterified cholesterol, and a molecule of the protein apolipoprotein B-48 (see p. 228). This layer stabilizes the particle and increases its solubility, thereby preventing multiple particles from coalescing. [Note:

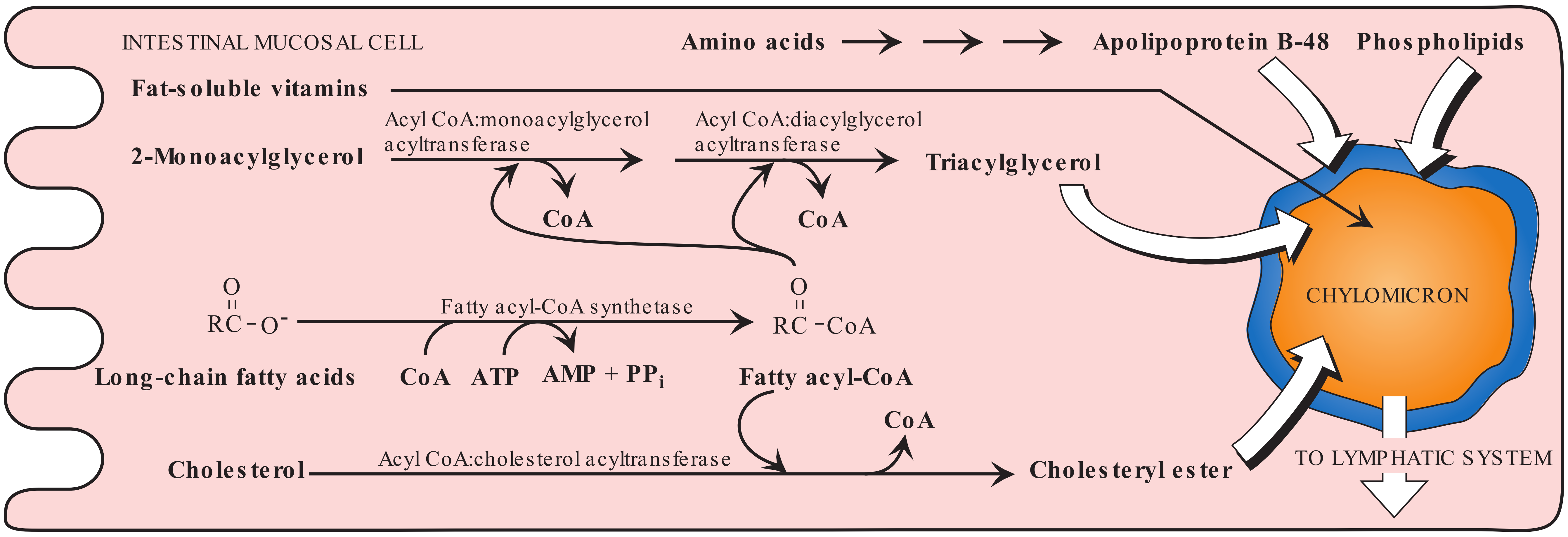

INTESTINAL MUCOSAL CELL
Amino acids
Apolipoprotein B-48
Phospholipids
Fat-soluble vitamins
Acyl CoA:monoacylglycerol acyltransferase
Acyl CoA:diacylglycerol acyltransferase
2-Monoacylglycerol
Triacylglycerol
CoA
CoA
$RC(=O)-O^-$
Fatty acyl-CoA synthetase
$RC(=O)-CoA$
Long-chain fatty acids
CoA
ATP
AMP + PP_i
Fatty acyl-CoA
CoA
Cholesterol
Acyl CoA:cholesterol acyltransferase
Cholesteryl ester
CHYLOMICRON
TO LYMPHATIC SYSTEM

Figure 15.6
Assembly and secretion of chylomicrons by intestinal mucosal cells. [Note: Short- and medium-chain length fatty acids do not require incorporation into micelles or chylomicrons and directly enter into the blood.]
CoA = coenzyme A; AMP = adenosine monophosphate; PP_i = pyrophosphate.

Microsomal triglyceride transfer protein is essential for the assembly of these (and other) TAG-rich apolipoprotein B–containing particles in the endoplasmic reticulum.] The lipoprotein particles are released by exocytosis from enterocytes into the lacteals (lymphatic vessels originating in the villi of the small intestine). The presence of these particles in the lymph after a lipid-rich meal gives it a milky appearance. This lymph is called chyle (as opposed to chyme, the name given to the semifluid mass of partially digested food that passes from the stomach to the duodenum), and the particles are named chylomicrons. Chylomicrons follow the lymphatic system to the thoracic duct and are then conveyed to the left subclavian vein, where they enter the blood. The steps in the production of chylomicrons are summarized in Figure 15.6. [Note: Once released into blood, chylomicrons pick up apolipoproteins E and C-II.] (For a more detailed description of chylomicron structure and metabolism, see p. 228.)

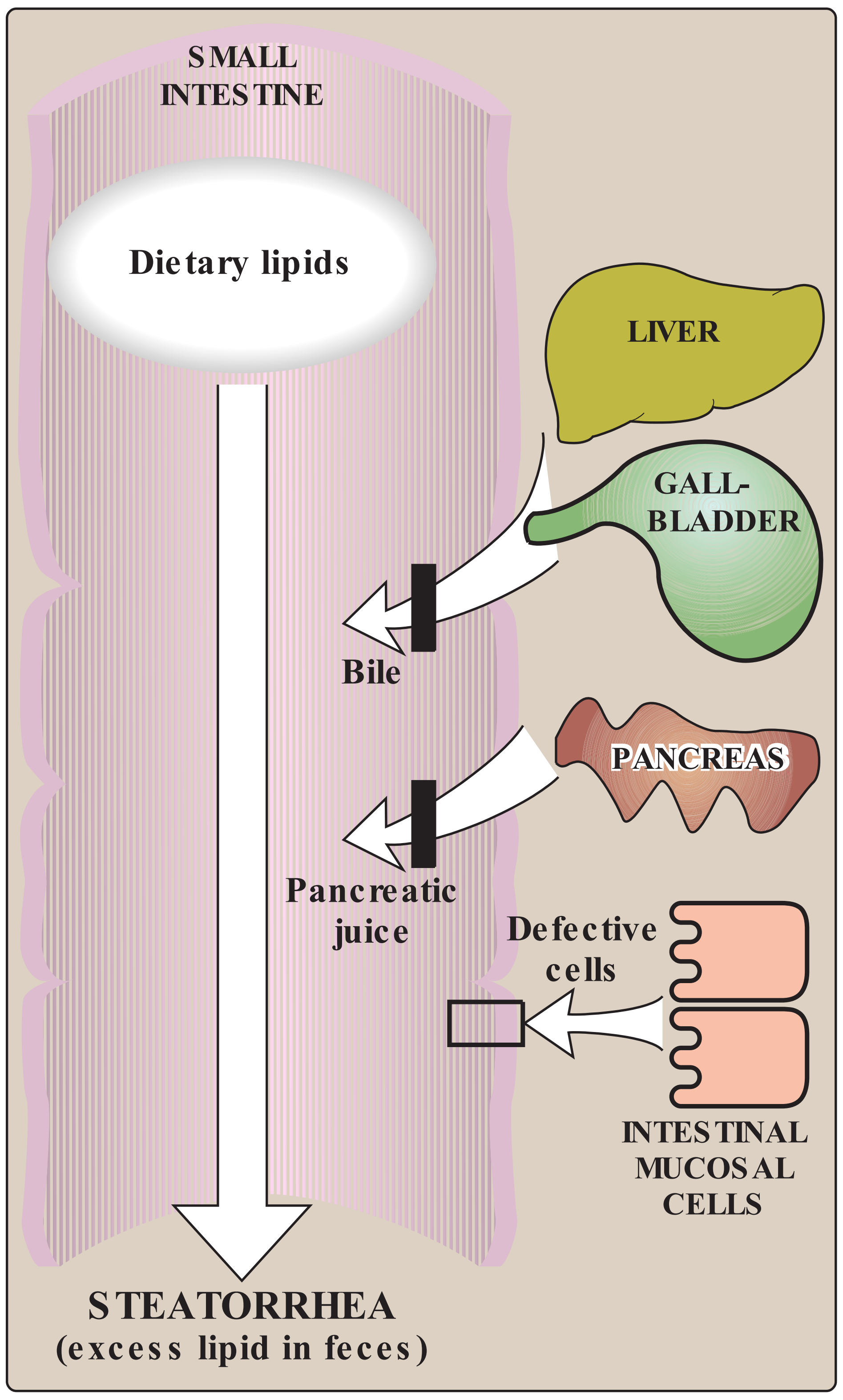


Figure 15.7
Possible causes of steatorrhea.

H. Use of dietary lipids by the tissues

TAG contained in chylomicrons is broken down primarily in the capillaries of skeletal and cardiac muscle and adipose tissues. TAG in chylomicrons is degraded to free fatty acids and glycerol by lipoprotein lipase (LPL). This enzyme is synthesized primarily by adipocytes and muscle cells. It is secreted and becomes associated with the luminal surface of endothelial cells in the capillary beds of the peripheral tissues. [Note: Familial LPL deficiency (type I hyperlipoproteinemia) is a rare, autosomal recessive disorder caused by a deficiency of LPL or its coenzyme apolipoprotein C-II (see p. 228). The result is fasting chylomicronemia and hypertriacylglycerolemia.]

1. **Fate of free fatty acids:** The free fatty acids derived from the hydrolysis of TAG may either directly enter adjacent muscle cells or adipocytes or be transported in the blood in association with serum albumin until they are taken up by cells. [Note: Serum albumin is a large glycoprotein secreted by the liver. It transports a number of primarily hydrophobic compounds in the circulation, including free fatty acids and some drugs.] Most cells can oxidize fatty acids to produce energy (see p. 190). Adipocytes can also reesterify free fatty acids to produce TAG molecules, which are stored until the fatty acids are needed by the body (see p. 188).

2. **Fate of glycerol:** Glycerol released from TAG is taken up from the blood and phosphorylated by hepatic glycerol kinase to produce glycerol 3-phosphate, which can enter either glycolysis or gluconeogenesis by oxidation to dihydroxyacetone phosphate (see p. 190).

3. **Fate of the remaining chylomicron components:** After most of the TAG has been removed, the chylomicron remnants (which contain cholesteryl esters, phospholipids, apolipoproteins, fat-soluble vitamins, and a small amount of TAG) bind to receptors on the liver (apolipoprotein E is the ligand; see p. 230) and are endocytosed. The intracellular remnants are hydrolyzed to their component parts. Cholesterol and the nitrogenous bases of phospholipids (for example, choline) can be recycled by the body. [Note: If removal of remnants by the liver is decreased due to impaired binding to their receptor, they accumulate in the plasma. This is seen in the rare type III hyperlipoproteinemia (also called familial dysbetalipoproteinemia, see p. 231).]

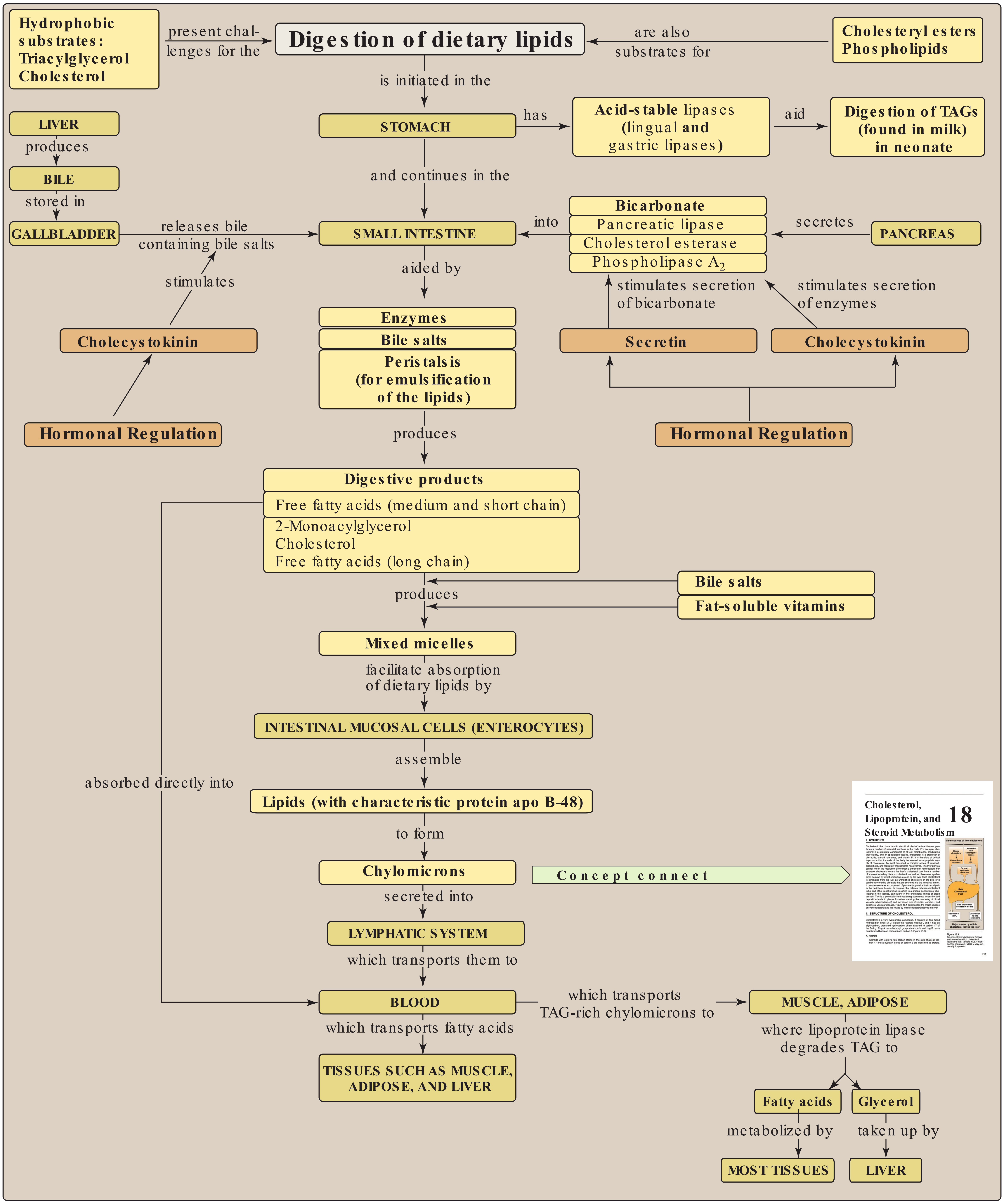

Hydrophobic substrates: Triacylglycerol Cholesterol
present challenges for the
Digestion of dietary lipids
are also substrates for
Cholesteryl esters Phospholipids
is initiated in the
STOMACH
has
Acid-stable lipases (lingual and gastric lipases)
aid
Digestion of TAGs (found in milk) in neonate
LIVER
produces
BILE
stored in
GALLBLADDER
releases bile containing bile salts
and continues in the
SMALL INTESTINE
into
Bicarbonate
Pancreatic lipase
Cholesterol esterase
Phospholipase A_2
secretes
PANCREAS
stimulates
aided by
Enzymes
Bile salts
Peristalsis (for emulsification of the lipids)
stimulates secretion of bicarbonate
stimulates secretion of enzymes
Cholecystokinin
Secretin
Cholecystokinin
Hormonal Regulation
Hormonal Regulation
produces
Digestive products
Free fatty acids (medium and short chain)
2-Monoacylglycerol
Cholesterol
Free fatty acids (long chain)
Bile salts
Fat-soluble vitamins
produces
Mixed micelles
facilitate absorption of dietary lipids by
INTESTINAL MUCOSAL CELLS (ENTEROCYTES)
absorbed directly into
assemble
Lipids (with characteristic protein apo B-48)
to form
Chylomicrons
Concept connect
Cholesterol, Lipoprotein, and Steroid Metabolism 18
secreted into
LYMPHATIC SYSTEM
which transports them to
BLOOD
which transports TAG-rich chylomicrons to
MUSCLE, ADIPOSE
which transports fatty acids
TISSUES SUCH AS MUSCLE, ADIPOSE, AND LIVER
where lipoprotein lipase degrades TAG to
Fatty acids
Glycerol
metabolized by
taken up by
MOST TISSUES
LIVER

Figure 15.8
Key concept map for metabolism of dietary lipids. apo = apolipoprotein; TAGs = triacylglycerols.

III. CHAPTER SUMMARY

The digestion of **dietary lipids** begins in the **stomach** and continues in the **small intestine** (Figure 15.8). The **hydrophobic nature** of lipids requires that the dietary lipids, particularly those that contain long-chain length fatty acids (LCFAs), be **emulsified** for efficient degradation. Triacylglycerols (TAG) obtained from milk contain **short-** to **medium-chain length fatty acids** that can be degraded in the **stomach** by the acid lipases (lingual lipase and gastric lipase). Cholesteryl esters (CEs), phospholipids (PLs), and TAG containing LCFAs are degraded in the **small intestine** by enzymes secreted by the **pancreas**. The most important of these enzymes are pancreatic lipase, phospholipase A_2, and cholesterol esterase. The dietary lipids are **emulsified** in the small intestine using **peristaltic action** and **bile salts**, which serve as detergents. The primary products resulting from enzymatic degradation of dietary lipid are **2-monoacylglycerol**, unesterified **cholesterol**, and **free fatty acids**. These compounds, plus the fat-soluble vitamins, form **mixed micelles** that facilitate the absorption of dietary lipids by **intestinal mucosal cells** (**enterocytes**). These cells resynthesize TAG, CE, and PL using LCFAs and also synthesize protein (**apolipoprotein B-48**), all of which are then assembled with the fat-soluble vitamins into lipoprotein particles called **chylomicrons**. Short- and medium-chain fatty acids enter blood directly. Chylomicrons are released into the **lymph**, which carries them to the **blood**, where their lipid core is degraded by lipoprotein lipase (with apolipoprotein C-II as the coenzyme) in muscle and adipose tissues. Thus, dietary lipids are made available to the peripheral tissues. Problems with fat absorption cause **steatorrhea**. A deficiency in the ability to degrade chylomicron components, or remove their remnants after TAG has been removed, results in accumulation of these particles in blood.

Fatty Acid, Ketone Body, and Triacylglycerol Metabolism

16

I. OVERVIEW

Fatty acids exist "free" in the body (that is, they are unesterified) and as fatty acyl esters in more complex molecules such as triacylglycerols (TAGs). Low levels of free fatty acids (FFAs) occur in all tissues, but substantial amounts can sometimes be found in the plasma, particularly during fasting. Plasma FFAs (transported on serum albumin) are in route from their point of origin (TAG of adipose tissue or circulating lipoproteins) to their site of consumption (most tissues). FFAs can be oxidized by many tissues, particularly liver and muscle, to provide energy and, in liver, to provide the substrate for ketone body synthesis. Fatty acids are also structural components of membrane lipids, such as phospholipids and glycolipids (see p. 201). Fatty acids attached to certain proteins enhance the ability of those proteins to associate with membranes (see p. 206). Fatty acids are also precursors of the hormone-like prostaglandins (see p. 213). Esterified fatty acids, in the form of TAGs stored in white adipose tissue (WAT), serve as the major energy reserve of the body. Alterations in fatty acid metabolism are associated with obesity and diabetes. Figure 16.1 illustrates the metabolic pathways of fatty acid synthesis and degradation and their relationship to carbohydrate metabolism.

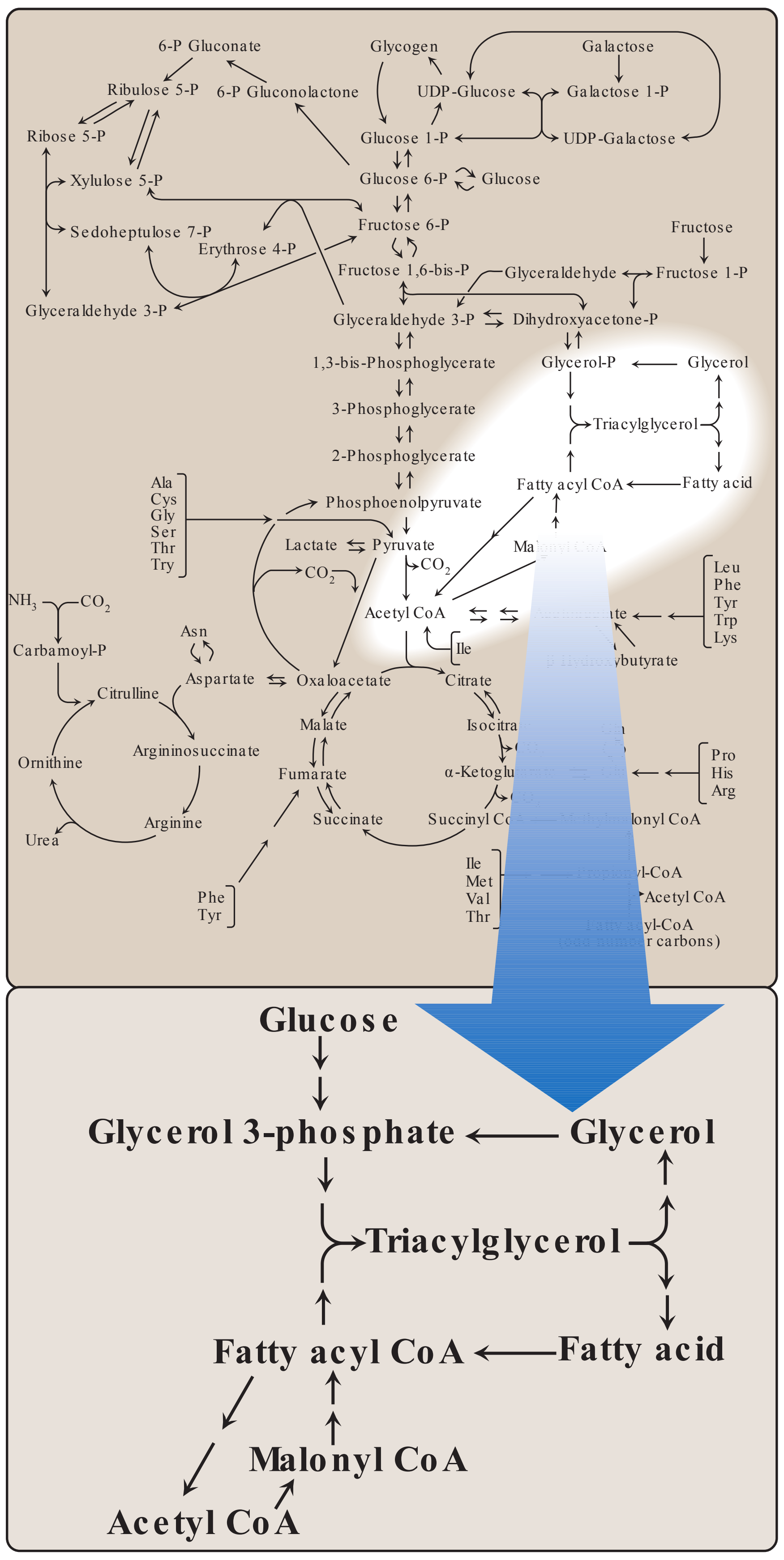

6-P Gluconate
Glycogen
Galactose
Ribulose 5-P
6-P Gluconolactone
UDP-Glucose
Galactose 1-P
Ribose 5-P
Glucose 1-P
UDP-Galactose
Xylulose 5-P
Glucose 6-P
Glucose
Sedoheptulose 7-P
Erythrose 4-P
Fructose 6-P
Fructose
Fructose 1,6-bis-P
Glyceraldehyde
Fructose 1-P
Glyceraldehyde 3-P
Glyceraldehyde 3-P
Dihydroxyacetone-P
1,3-bis-Phosphoglycerate
Glycerol-P
Glycerol
3-Phosphoglycerate
Triacylglycerol
2-Phosphoglycerate
Fatty acyl CoA
Fatty acid
Ala
Cys
Gly
Ser
Thr
Try
Phosphoenolpyruvate
Lactate
Pyruvate
CO_2
CO_2
Leu
Phe
Tyr
Trp
Lys
NH_3
CO_2
Acetyl CoA
Carbamoyl-P
Asn
Ile
Aspartate
Oxaloacetate
Citrate
Citrulline
Malate
Ornithine
Argininosuccinate
Fumarate
α-Ketoglu
Pro
His
Arg
Arginine
Succinate
Urea
Ile
Met
Val
Thr
Acetyl CoA
Phe
Tyr
Glucose
Glycerol 3-phosphate
Glycerol
Triacylglycerol
Fatty acyl CoA
Fatty acid
Malonyl CoA
Acetyl CoA

Figure 16.1
Triacylglycerol synthesis and degradation. CoA = coenzyme A.

II. STRUCTURE OF FATTY ACIDS

A fatty acid consists of a hydrophobic hydrocarbon chain with a terminal carboxyl group that has a pK_a of about 4.8 (Figure 16.2). At physiologic pH, the terminal carboxyl group (–COOH) ionizes, becoming $–COO^-$. [Note: When the pH is above the pK, the deprotonated form predominates (see p.7).] This anionic group has an affinity for water, giving the fatty acid its amphipathic nature (having both a hydrophilic and a hydrophobic region). However, for long-chain fatty acids (LCFAs), the hydrophobic portion is predominant. These molecules are highly water insoluble and must be transported in the circulation in association with protein. More than 90% of the fatty acids found in plasma are in the form of fatty acid esters (primarily TAG, cholesteryl esters, and phospholipids) contained in circulating lipoprotein particles (see p. 227). Unesterified (free) fatty acids are transported in the circulation in association with albumin, the most abundant protein in serum.

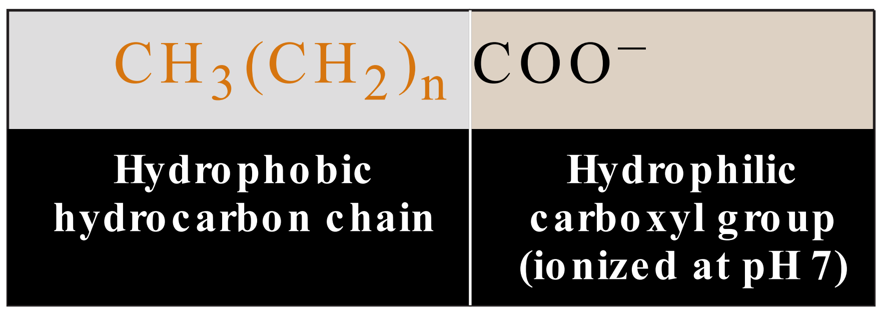

$CH_3(CH_2)_n$ COO^-
Hydrophobic hydrocarbon chain
Hydrophilic carboxyl group (ionized at pH 7)

Figure 16.2
Structure of a fatty acid.

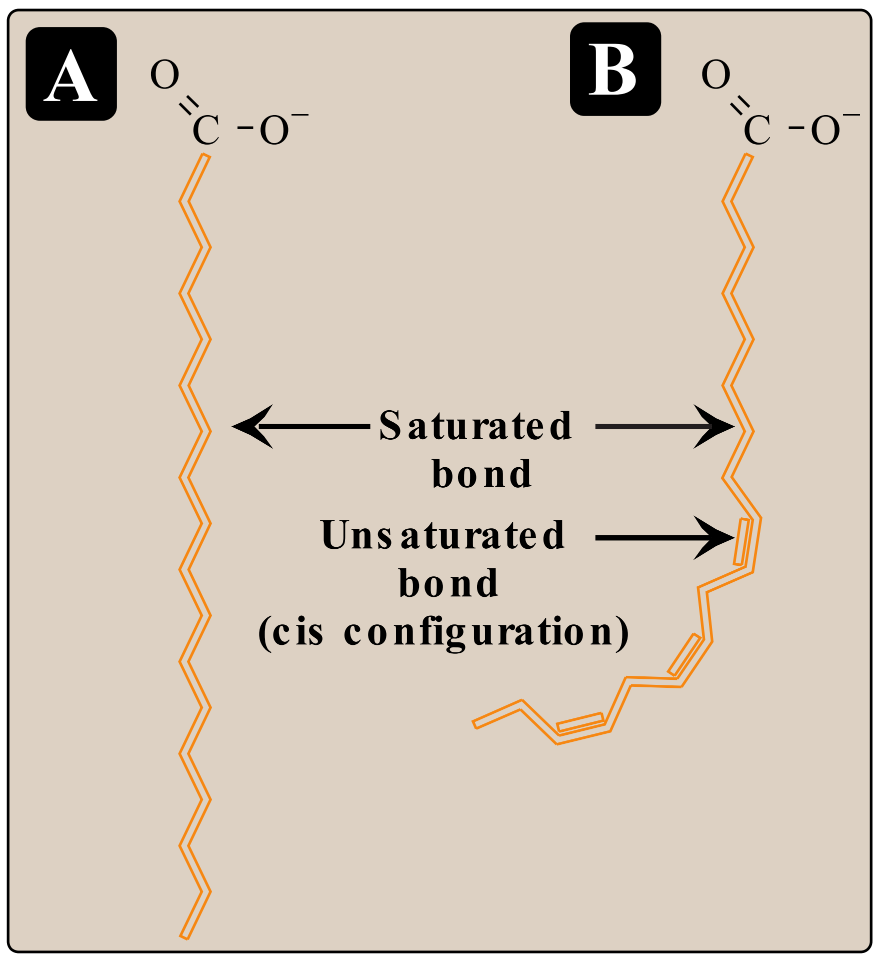


Figure 16.3
A saturated (A) and an unsaturated (B) fatty acid. Orange denotes hydrophobic portions of the molecules. [Note: Cis double bonds cause a fatty acid to "kink."]

A. Saturation of fatty acids

Fatty acid chains may contain no double bonds (that is, be saturated) or contain one or more double bonds (that is, be mono- or polyunsaturated). In humans, the majority are saturated or monounsaturated. When double bonds are present, they are nearly always in the cis rather than in the trans configuration. (See p. 363 for a discussion of the dietary occurrence of cis and trans unsaturated fatty acids.) The introduction of a cis double bond causes the fatty acid to bend or "kink" at that position (Figure 16.3). If the fatty acid has two or more double bonds, they are always spaced at three-carbon intervals. [Note: In general, addition of double bonds decreases the melting temperature (T_m) of a fatty acid, whereas increasing the chain length increases the T_m. Because membrane lipids typically contain LCFAs, the presence of double bonds in some fatty acids helps maintain the fluid nature of those lipids.]

Fatty acids with chain lengths of 4 to 10 carbons are found in significant quantities in milk.

Structural lipids and triacylglycerols contain primarily fatty acids of at least 16 carbons.

COMMON NAME	STRUCTURE
Formic acid	1
Acetic acid	2:0
Propionic acid	3:0
Butyric acid	4:0
Capric acid	10:0
Palmitic acid	16:0
Palmitoleic acid	16:1(9)
Stearic acid	18:0
Oleic acid	18:1(9)
Linoleic acid	18:2(9,12)
α-Linolenic acid	18:3(9,12,15)
Arachidonic acid	20:4(5, 8,11,14)
Lignoceric acid	24:0
Nervonic acid	24:1(15)

Precursor of prostaglandins

Essential fatty acids

Figure 16.4
Some fatty acids of physiologic importance. [Note: If a fatty acid has 2–4 carbons, it is considered short; if 6–12, medium; if 14–20, long; and if 22 or more, very long.]

B. Chain lengths of fatty acids and positions of double bonds

The common names and structures of some fatty acids of physiologic importance are listed in Figure 16.4. In humans, fatty acids with an even number of carbon atoms (16, 18, or 20) predominate, with longer fatty acids (over 22 carbons) being found in the brain. The carbon atoms are numbered, beginning with the carboxyl carbon as carbon 1. The number before the colon indicates the number of carbons in the chain, and those after the colon indicate the numbers and positions (relative to the carboxyl end) of double bonds. For example, as denoted in Figure 16.4, arachidonic acid, 20:4(5,8,11,14), is 20 carbons long and has 4 double bonds (between carbons 5–6, 8–9, 11–12, and 14–15). [Note: Carbon 2, the carbon to which the carboxyl group is attached, is also called the α-carbon, carbon 3 is the β-carbon, and carbon 4 is the γ-carbon. The carbon of the terminal methyl group is called the ω-carbon regardless of the chain length.] The double bonds in a fatty acid can also be denoted relative to the ω (or methyl) end of the chain. Arachidonic acid is referred to as an ω-6 fatty acid acid (also an n-6 fatty acid, Figure 16.5A) because the terminal double bond is six bonds in from the ω end (Figure 16.5B). Another ω-6 fatty acid is the essential linoleic acid 18:2(9,12). In contrast, α-linolenic acid, 18:3(9,12,15), is an essential ω-3 fatty acid. (See p. 363 for a discussion of the nutritional significance of ω-3 and ω-6 fatty acids.)

C. Essential fatty acids

Linoleic acid, the precursor of ω-6 arachidonic acid, which is the substrate for prostaglandin synthesis (see p. 213), and α-linolenic acid, the precursor of ω-3 fatty acids that are important for growth and development, are dietary essentials in humans because we lack the enzymes needed to synthesize them. Plants provide us with these essential fatty acids. [Note: Arachidonic acid becomes essential if linoleic acid is deficient in the diet.]

Essential fatty acid deficiency (rare) can result in a dry, scaly dermatitis as a result of an inability to synthesize molecules that provide the water barrier in skin (see p. 206).

III. DE NOVO SYNTHESIS OF FATTY ACIDS

A large proportion of the fatty acids used by the body is supplied by the diet. Carbohydrates and protein obtained from the diet in excess of the body's needs for these compounds can be converted to fatty acids, which are stored as TAGs. (See p. 321 for a discussion of the metabolism of dietary nutrients in the well-fed state.) In adult humans, fatty acid synthesis occurs primarily in the liver and lactating mammary glands and, to a lesser extent, in adipose tissue. This cytosolic process incorporates carbons from acetyl coenzyme A (CoA) into the growing fatty acid chain, using adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide phosphate (NADPH).

A. Production of cytosolic acetyl coenzyme A

The first step in de novo fatty acid synthesis is the transfer of acetate units from mitochondrial acetyl CoA to the cytosol. Mitochondrial acetyl CoA is produced by the oxidation of pyruvate (see p. 109) and by the catabolism of certain amino acids (see p. 266). The CoA portion of acetyl CoA, however, cannot cross the inner mitochondrial membrane, and only the acetyl portion enters the cytosol. It does so as part of citrate produced by the condensation of acetyl CoA with oxaloacetate (OAA) by citrate synthase (Figure 16.6). [Note: The translocation of citrate to the cytosol occurs when the mitochondrial citrate concentration is high. This is observed when isocitrate dehydrogenase of the citric acid cycle is inhibited by the presence of large amounts of ATP, causing citrate and isocitrate to accumulate (see p. 112). Therefore, cytosolic citrate may be viewed as a high-energy signal. Because a large amount of ATP is needed for fatty acid synthesis, the increase in both ATP and citrate enhances this pathway.]

B. Carboxylation of acetyl coenzyme A to malonyl coenzyme A

The energy for the carbon-to-carbon condensations in fatty acid synthesis is supplied by the process of carboxylation followed by decarboxylation of acyl groups in the cytosol. The carboxylation of acetyl CoA to form malonyl CoA is catalyzed by acetyl CoA carboxylase (ACC) (Figure 16.7), and requires CO_2 and ATP. The coenzyme is the vitamin biotin, which is covalently bound to a lysyl residue of the carboxylase (see Figure 28.16, p. 381). ACC carboxylates the bound biotin, which transfers the activated carboxyl group to acetyl CoA.

1. **Short-term regulation of acetyl coenzyme A carboxylase:** This carboxylation is both the rate-limiting and the regulated step in fatty acid synthesis (see Figure 16.7). The inactive form of ACC is a protomer. The enzyme undergoes allosteric activation by citrate, which causes protomers to polymerize, and allosteric inactivation by long-chain fatty acyl CoA (the end product of the pathway), which causes depolymerization. A second mechanism of short-term regulation is by reversible phosphorylation. Adenosine monophosphate–activated protein kinase (AMPK) phosphorylates and inactivates ACC. AMPK itself is allosterically activated by AMP and covalently activated by phosphorylation via several kinases. At least one of these AMPK kinases is activated by cAMP-dependent protein kinase A (PKA). Thus, in the presence of counterregulatory hormones, such as epinephrine

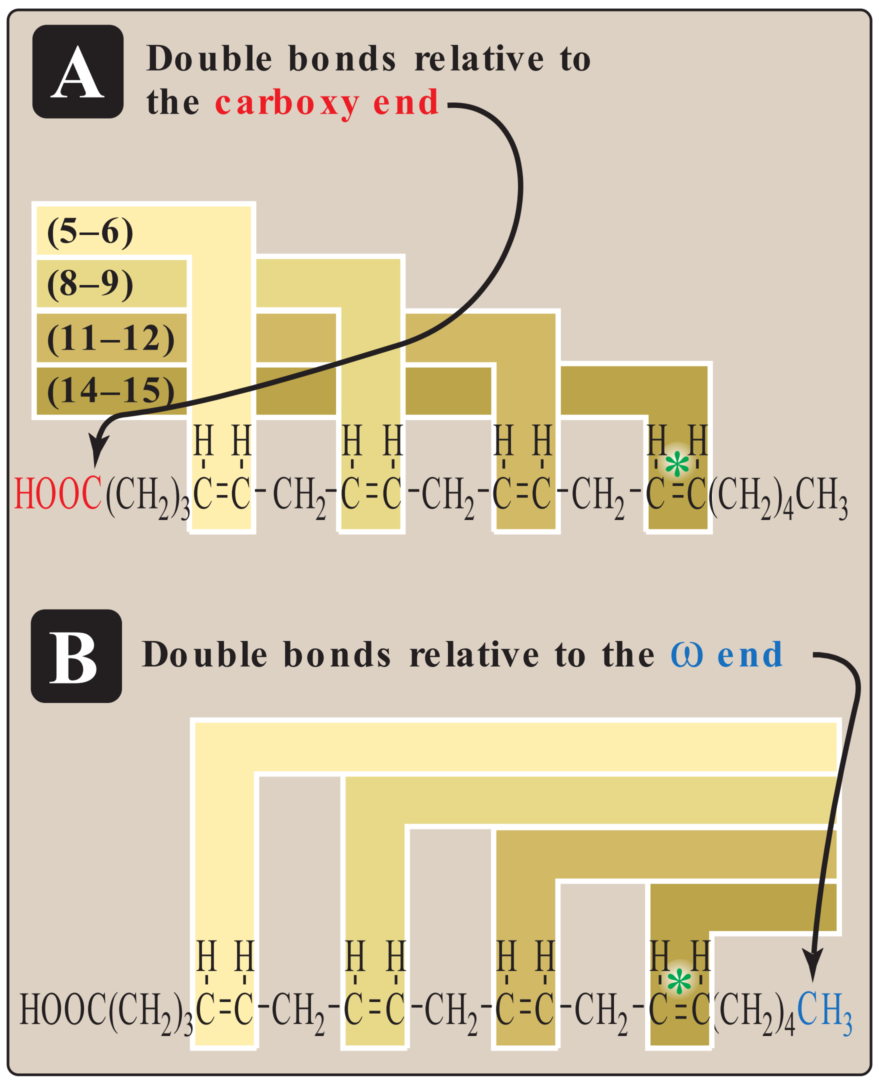

A
Double bonds relative to the carboxy end
(5–6)
(8–9)
(11–12)
(14–15)
H H H H H H H H
HOOC(CH2)3C=C-CH2-C=C-CH2-C=C-CH2-C=C(CH2)4CH3
*
B
Double bonds relative to the ω end
H H H H H H H H
HOOC(CH2)3C=C-CH2-C=C-CH2-C=C-CH2-C=C(CH2)4CH3
*

Figure 16.5
Arachidonic acid, illustrating the position of the double bonds. Arachidonic acid, 20:4(5,8,11,14) is an n-6 fatty acid because the double bond furthest from the carboxy end (carbon 1) is 14 carbons from that end: 20 – 14 = 6. It is also referred to as an ω-6 fatty acid because the terminal double bond is six bonds in from the ω end. Thus, the "ω" and "n" designations are equivalent (see *).

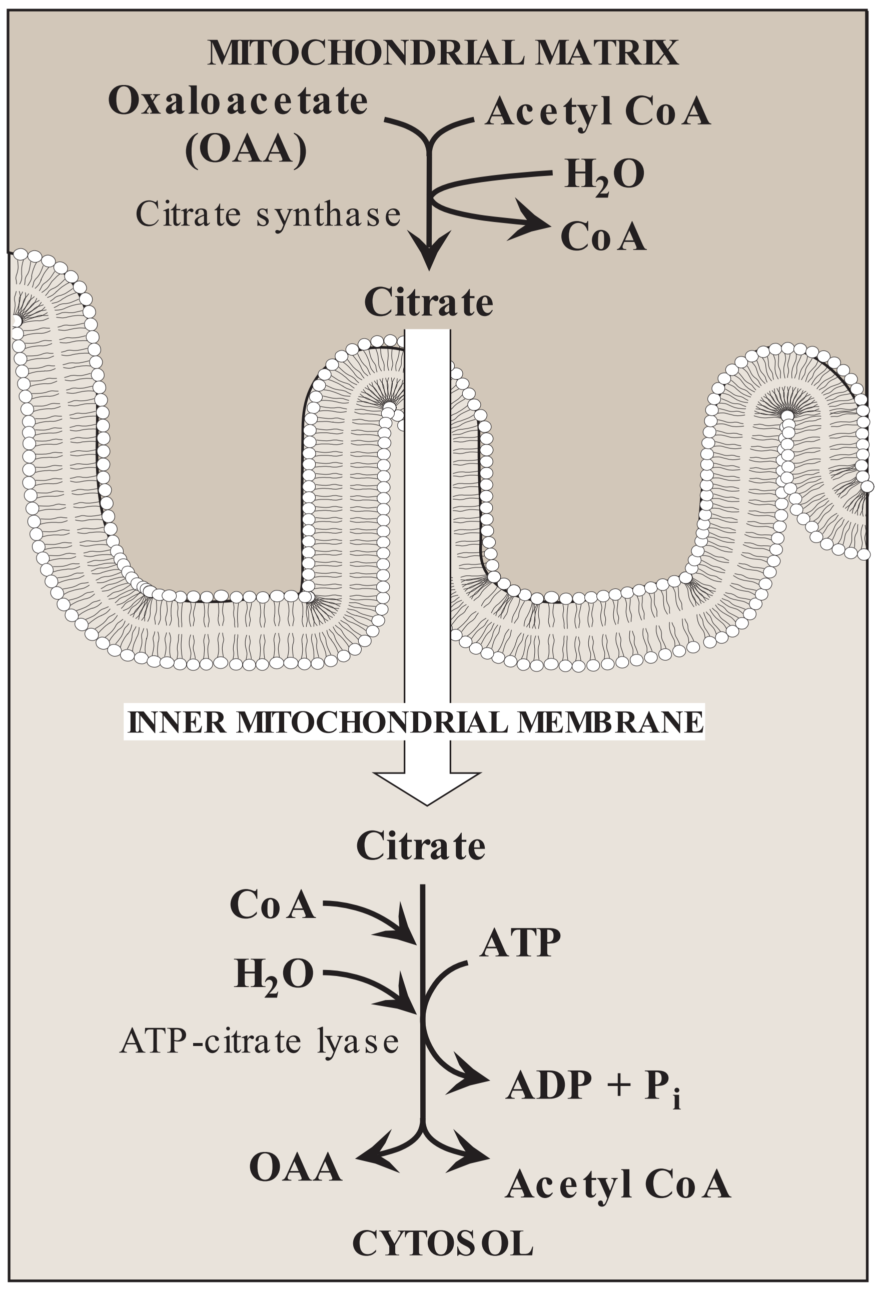


Figure 16.6
Production of cytosolic acetyl coenzyme A (CoA). Citrate is transported by the tricarboxylate transporter system. ADP = adenosine monophosphate; P_i = inorganic phosphate.

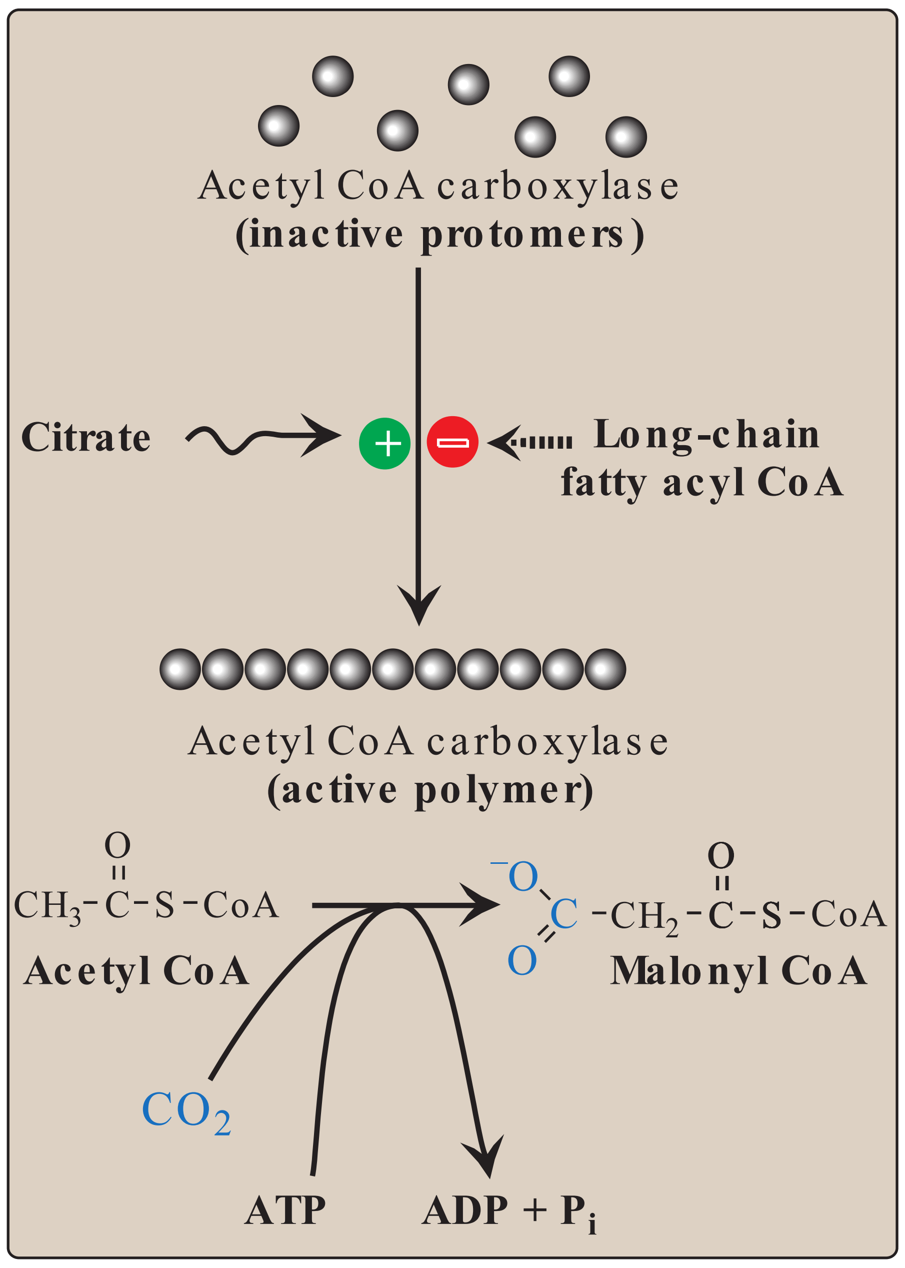

Acetyl CoA carboxylase
(inactive protomers)
Citrate
Long-chain fatty acyl CoA
Acetyl CoA carboxylase
(active polymer)
$CH_3-\overset{O}{\overset{\|}{C}}-S-CoA$
Acetyl CoA
$^-O_2C-CH_2-\overset{O}{\overset{\|}{C}}-S-CoA$
Malonyl CoA
CO$_2$
ATP
ADP + P$_i$

Figure 16.7
Allosteric regulation of malonyl coenzyme A (CoA) synthesis by acetyl CoA carboxylase. The carboxyl group contributed by dissolved CO$_2$ is shown in blue. P$_i$ = inorganic phosphate; ADP = adenosine diphosphate.

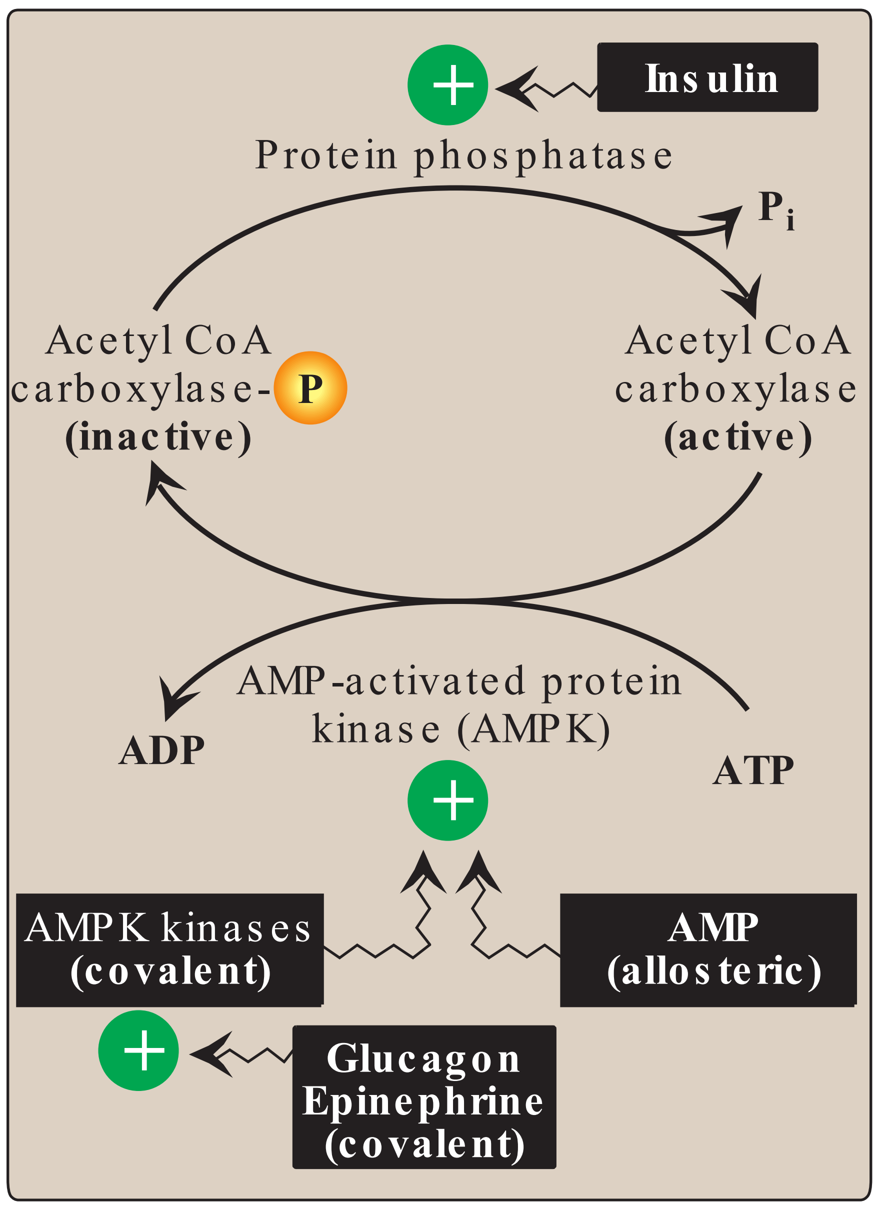

Insulin
Protein phosphatase
P$_i$
Acetyl CoA carboxylase-P
(inactive)
Acetyl CoA carboxylase
(active)
AMP-activated protein kinase (AMPK)
ADP
ATP
AMPK kinases
(covalent)
AMP
(allosteric)
Glucagon
Epinephrine
(covalent)

Figure 16.8
Covalent regulation (phosphorylation) of acetyl CoA carboxylase by AMPK, which itself is regulated both covalently and allosterically. CoA = coenzyme A; ADP = adenosine diphosphate; Ⓟ = phosphate; P$_i$ = inorganic phosphate; AMP = adenosine monophosphate.

and glucagon, ACC is phosphorylated and, thereby, inactivated (Figure 16.8). In the presence of insulin, ACC is dephosphorylated and, thereby, activated. [Note: This is analogous to the regulation of glycogen synthase (see p. 131).]

2. **Long-term regulation of acetyl coenzyme A carboxylase:** Prolonged consumption of a diet containing excess calories (particularly high-calorie, high-carbohydrate diets) causes an increase in ACC synthesis, thereby increasing fatty acid synthesis. Conversely, a low-calorie or a high-fat diet causes a reduction in fatty acid synthesis by decreasing ACC synthesis. [Note: Synthesis of the carboxylase is upregulated by insulin via a sterol regulatory element–binding protein, SREBP-1. The function and regulation of SREBPs are described on p. 222. Fatty acid synthase (see below) is similarly regulated by diet and SREBP-1.] Metformin, used in the treatment of type 2 diabetes, lowers serum TAG through activation of AMPK, resulting in inhibition of ACC activity (by phosphorylation) and inhibition of ACC and fatty acid synthase expression (by decreasing SREBP-1). Metformin also lowers blood glucose by increasing AMPK-mediated uptake of glucose by muscle.

C. Fatty acid synthase: a multifunctional enzyme in eukaryotes

The remaining series of reactions of fatty acid synthesis in eukaryotes is catalyzed by the multifunctional, dimeric enzyme, fatty acid synthase (FAS). Each FAS monomer is a multicatalytic polypeptide with seven different enzymic domains plus a domain that covalently binds a molecule of 4'-phosphopantetheine. [Note: 4'-Phosphopantetheine, a derivative of the vitamin pantothenic acid (see p. 381), carries acyl units on its terminal thiol (–SH) group during fatty acid synthesis. It also is a component of CoA.] In prokaryotes, FAS is a multienzyme complex, and the 4'-phosphopantetheine domain is a separate protein, referred to as the acyl carrier protein (ACP). ACP is used to refer to the phosphopantetheine-containing domain of eukaryotic FAS. The reaction numbers in brackets below refer to Figure 16.9.

[1] An acetyl group is transferred from acetyl CoA to the –SH group of the ACP. Domain: Acetyl CoA-ACP acetyltransacylase.

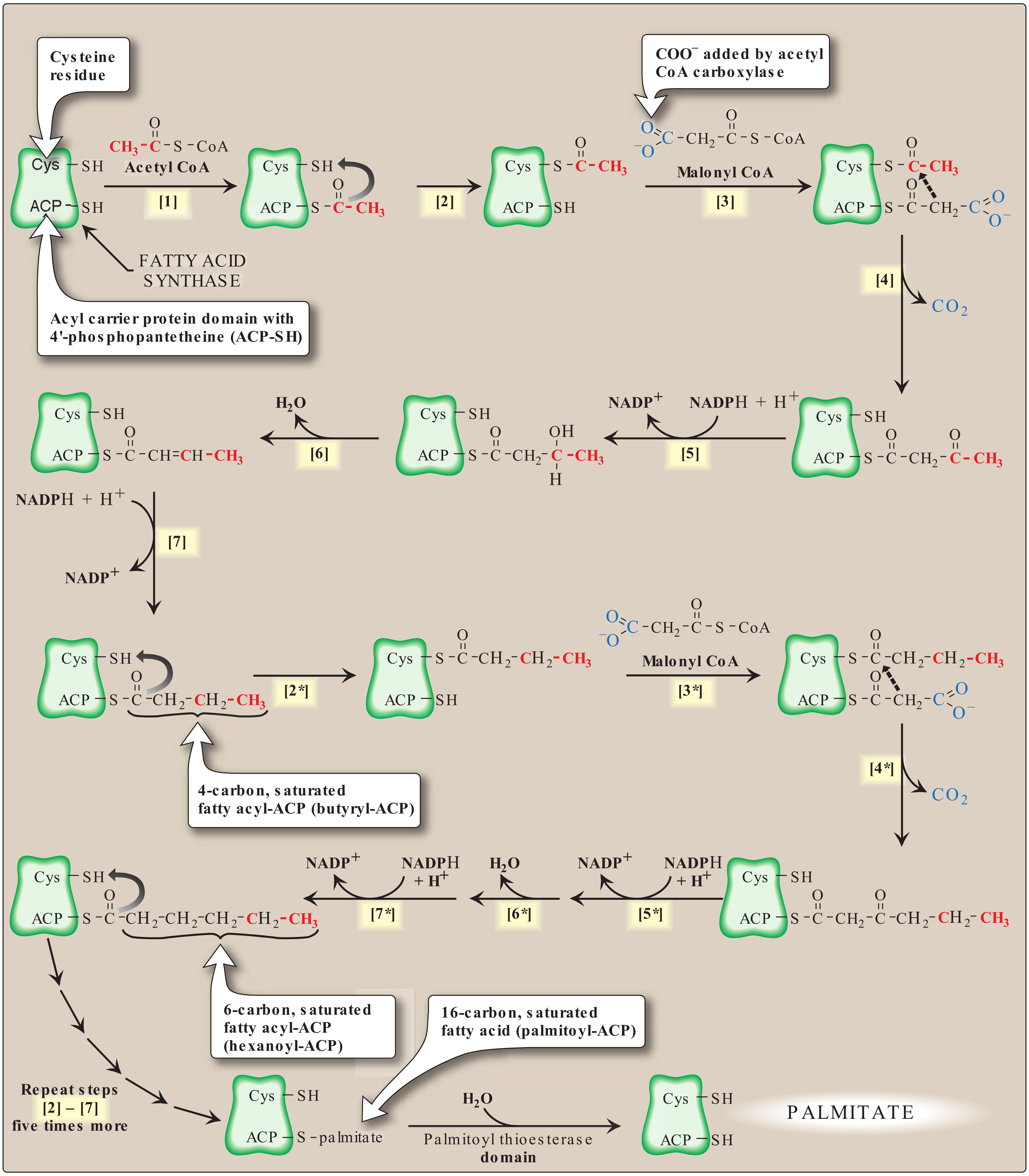
[2] Next, this two-carbon fragment is transferred to a temporary holding site, the thiol group of a cysteine residue on the enzyme.

[3] The now-vacant ACP accepts a three-carbon malonyl group from malonyl CoA. Domain: Malonyl CoA-ACP transacylase.

[4] The acetyl group on the cysteine residue condenses with the malonyl group on ACP as the CO$_2$ originally added by acetyl CoA carboxylase is released. The result is a four-carbon unit attached to the ACP domain. The loss of free energy from the decarboxylation drives the reaction. Domain: 3-Ketoacyl-ACP synthase, also known as "condensing enzyme."

The next three reactions convert the 3-ketoacyl group to the corresponding saturated acyl group by a pair of NADPH-requiring reductions and a dehydration step.

[5] The keto group is reduced to an alcohol. Domain: 3-Ketoacyl-ACP reductase.



Cysteine residue

COO⁻ added by acetyl CoA carboxylase

Acyl carrier protein domain with 4'-phosphopantetheine (ACP-SH)

4-carbon, saturated fatty acyl-ACP (butyryl-ACP)

6-carbon, saturated fatty acyl-ACP (hexanoyl-ACP)

16-carbon, saturated fatty acid (palmitoyl-ACP)

Repeat steps [2] – [7] five times more

Cys
SH
ACP
SH
$CH_3-C(=O)-S-CoA$
Acetyl CoA
[1]
[2]
Malonyl CoA
[3]
FATTY ACID SYNTHASE
[4]
CO_2
NADP$^+$
NADPH + H$^+$
[5]
H_2O
[6]
NADPH + H$^+$
[7]
NADP$^+$
[2*]
Malonyl CoA
[3*]
[4*]
CO_2
[5*]
[6*]
[7*]
S-palmitate
H_2O
Palmitoyl thioesterase domain
PALMITATE

Figure 16.9

Synthesis of palmitate (16:0) by multifunctional fatty acid synthase (FAS). [Note: Numbers in brackets correspond to bracketed numbers in the text. A second repetition of the steps is indicated by numbers with an asterisk (*). Carbons provided directly by acetyl coenzyme A (CoA) are shown in red.] Cys = cysteine; ACP = acyl carrier protein domain; NADP(H) = nicotinamide adenine dinucleotide phosphate.

[6] A molecule of water is removed, creating a double bond between carbons 2 and 3 (the α- and β-carbons). Domain: 3-Hydroxyacyl-ACP dehydratase.

[7] The double bond is reduced. Domain: Enoyl-ACP reductase.

The result of these seven steps is production of a four-carbon compound (butyryl) whose three terminal carbons are fully saturated, and which remains attached to the ACP domain. These seven steps are repeated, beginning with the transfer of the butyryl chain from the ACP to the cysteine residue [2*], the attachment of a molecule of malonate to the ACP [3*], and the condensation of the two molecules liberating CO_2 [4*]. The carbonyl group at the β-carbon (carbon 3, the third carbon from the sulfur) is then reduced [5*], dehydrated [6*], and reduced [7*], generating hexanoyl-ACP. This cycle of reactions is repeated five more times, each time incorporating a two-carbon unit (derived from malonyl CoA) into the growing fatty acid chain at the carboxyl end. When the fatty acid reaches a length of 16 carbons, the synthetic process is terminated with palmitoyl-S-ACP. [Note: Shorter-length fatty acids are important end products in the lactating mammary gland.] Palmitoyl thioesterase, the final catalytic activity of FAS, cleaves the thioester bond, releasing a fully saturated molecule of palmitate (16:0). [Note: All the carbons in palmitic acid have passed through malonyl CoA except the two donated by the original acetyl CoA, which are found at the methyl (ω) end of the fatty acid. This underscores the rate-limiting nature of the ACC reaction.]

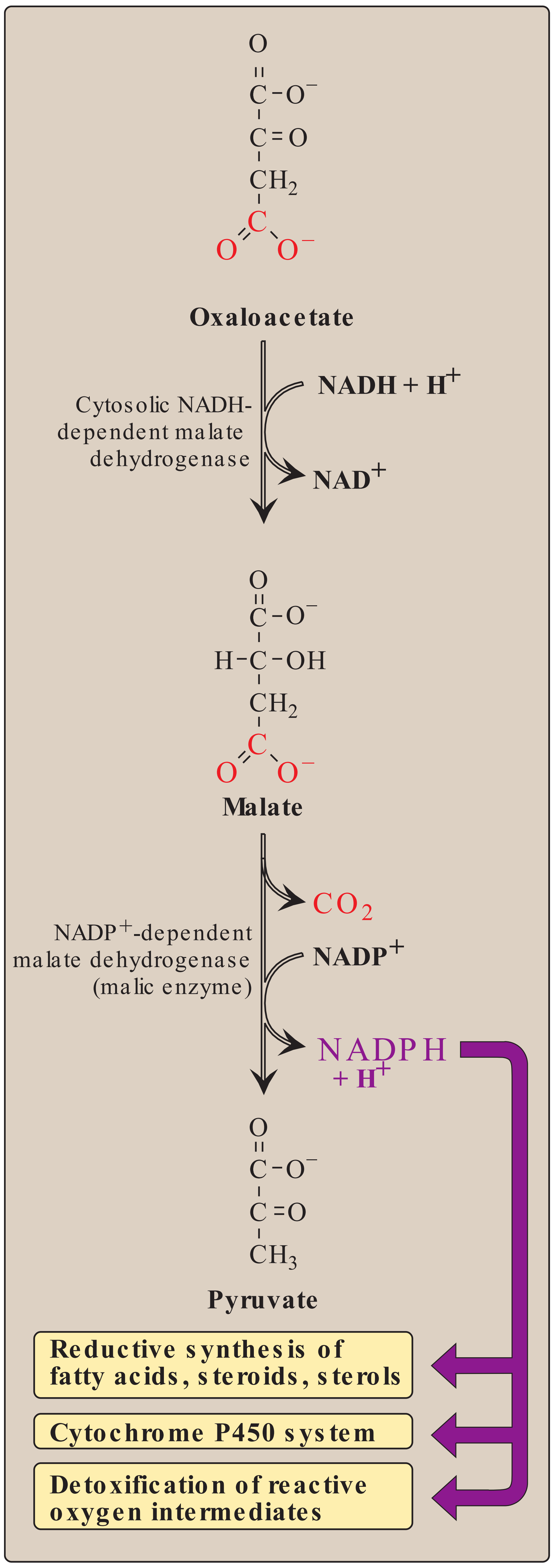

Oxaloacetate
Cytosolic NADH-dependent malate dehydrogenase
NADH + H+
NAD+
Malate
CO2
NADP+-dependent malate dehydrogenase (malic enzyme)
NADP+
NADPH + H+
Pyruvate
Reductive synthesis of fatty acids, steroids, sterols
Cytochrome P450 system
Detoxification of reactive oxygen intermediates

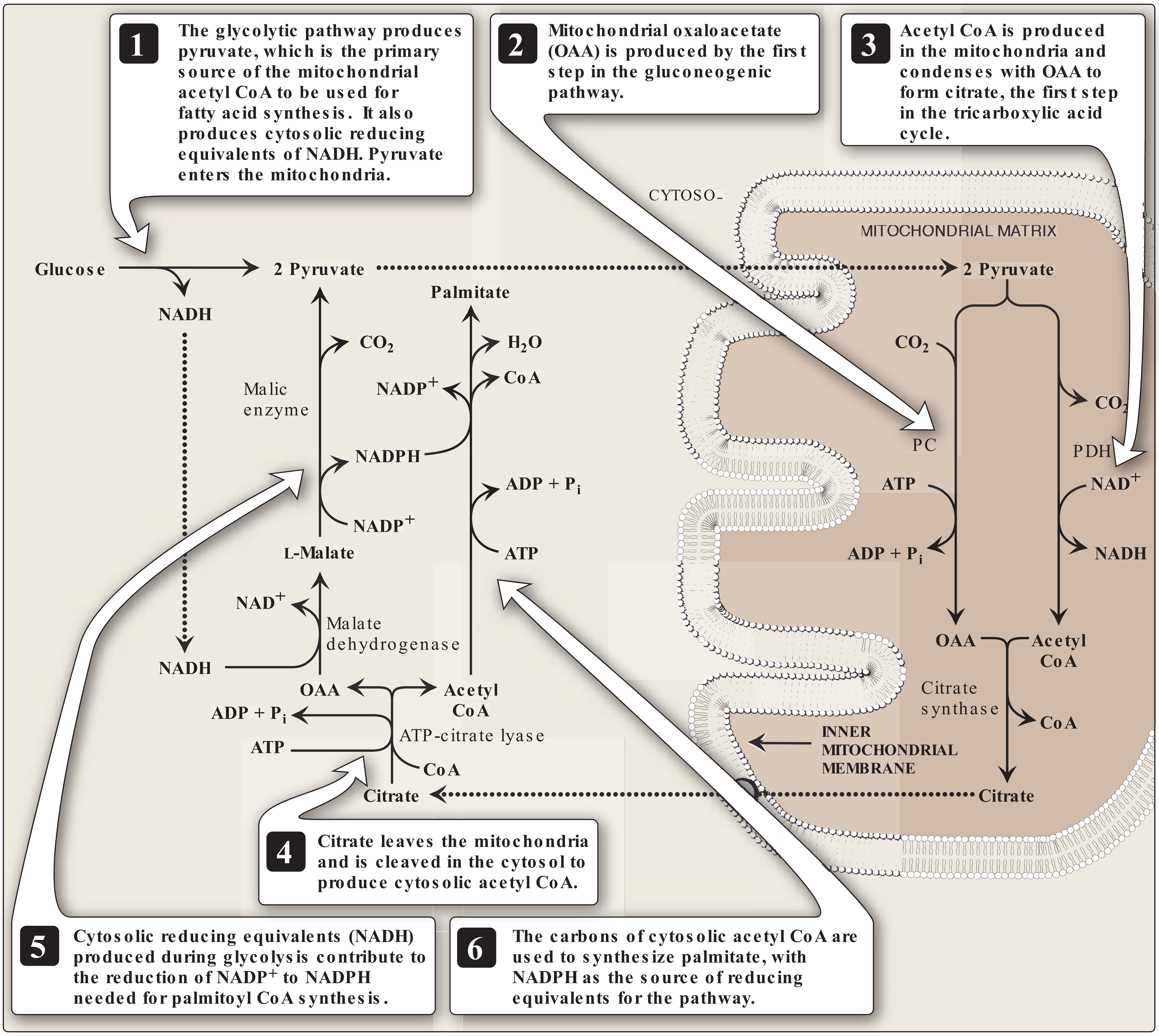
Figure 16.10
Cytosolic conversion of oxaloacetate to pyruvate with the generation of nicotinamide adenine dinucleotide phosphate (NADPH). [Note: The pentose phosphate pathway is also a source of NADPH.] NAD(H) = nicotinamide adenine dinucleotide.

D. Major sources of the reductant required for fatty acid synthesis

The pentose phosphate pathway (see p. 145) is a major supplier of NADPH, the reducatant required for fatty acid synthesis. Two NADPH are produced for each molecule of glucose that enters this pathway. The cytosolic conversion of malate to pyruvate, in which malate is oxidized and decarboxylated by cytosolic malic enzyme (NADP$^+$-dependent malate dehydrogenase), also produces cytosolic NADPH (and CO_2) as shown in Figure 16.10. [Note: Malate can arise from the reduction of OAA by cytosolic NADH-dependent malate dehydrogenase (see Figure 16.10). One source of the cytosolic NADH required for this reaction is that produced during glycolysis (see p. 101). OAA, in turn, can arise from citrate. Recall from Figure 16.6 that citrate, formed from OAA and acetyl CoA by citrate synthase, was shown to move from the mitochondria into the cytosol, where it is cleaved into acetyl CoA and OAA by ATP-citrate lyase.] A summary of the interrelationship between glucose metabolism and palmitate synthesis is shown in Figure 16.11.

E. Further elongation of fatty acid chains

Although palmitate, a 16-carbon, fully saturated LCFA (16:0), is the primary end product of fatty acid synthase activity, it can be further elongated by the addition of two-carbon units to the carboxylate end in the smooth endoplasmic reticulum (SER). Elongation requires a system of separate enzymes rather than a multifunctional enzyme. Malonyl CoA is the two-carbon donor, and NADPH supplies the electrons. The brain has additional elongation capabilities, allowing it to produce the very-long-chain fatty acids ([VLCFAs] over 22 carbons) that are required for synthesis of brain lipids.


CYTOSOL
MITOCHONDRIAL MATRIX
Glucose
NADH
2 Pyruvate
Palmitate
CO_2
H_2O
CoA
Malic enzyme
$NADP^+$
NADPH
$ADP + P_i$
$NADP^+$
L-Malate
ATP
NAD^+
Malate dehydrogenase
NADH
OAA
Acetyl CoA
$ADP + P_i$
ATP
ATP-citrate lyase
CoA
Citrate
2 Pyruvate
CO_2
CO_2
PC
PDH
ATP
NAD^+
$ADP + P_i$
NADH
OAA
Acetyl CoA
Citrate synthase
CoA
INNER MITOCHONDRIAL MEMBRANE
Citrate

1 The glycolytic pathway produces pyruvate, which is the primary source of the mitochondrial acetyl CoA to be used for fatty acid synthesis. It also produces cytosolic reducing equivalents of NADH. Pyruvate enters the mitochondria.

2 Mitochondrial oxaloacetate (OAA) is produced by the first step in the gluconeogenic pathway.

3 Acetyl CoA is produced in the mitochondria and condenses with OAA to form citrate, the first step in the tricarboxylic acid cycle.

4 Citrate leaves the mitochondria and is cleaved in the cytosol to produce cytosolic acetyl CoA.

5 Cytosolic reducing equivalents (NADH) produced during glycolysis contribute to the reduction of $NADP^+$ to NADPH needed for palmitoyl CoA synthesis.

6 The carbons of cytosolic acetyl CoA are used to synthesize palmitate, with NADPH as the source of reducing equivalents for the pathway.

Figure 16.11
Interrelationship between glucose metabolism and palmitate synthesis. CoA = coenzyme A; NAD(H) = nicotinamide adenine nucleotide; NADP(H) =nicotinamide adenine dinucleotide phosphate; ADP = adenosine diphosphate; P_i = inorganic phosphate; PC = pyruvate carboxylase; PDH = pyruvate dehydrogenase.

F. Desaturation of fatty acid chains

Enzymes (desaturases) also present in the SER are responsible for desaturating LCFAs (that is, adding cis double bonds). The desaturation reactions require O_2, NADH, cytochrome b_5, and its FAD-linked reductase. The fatty acid and the NADH get oxidized as the O_2 gets reduced to H_2O. The first double bond is typically inserted between carbons 9 and 10, producing primarily oleic acid, 18:1(9), and small amounts of palmitoleic acid, 16:1(9). A variety of polyunsaturated fatty acids can be made through additional desaturation combined with elongation.

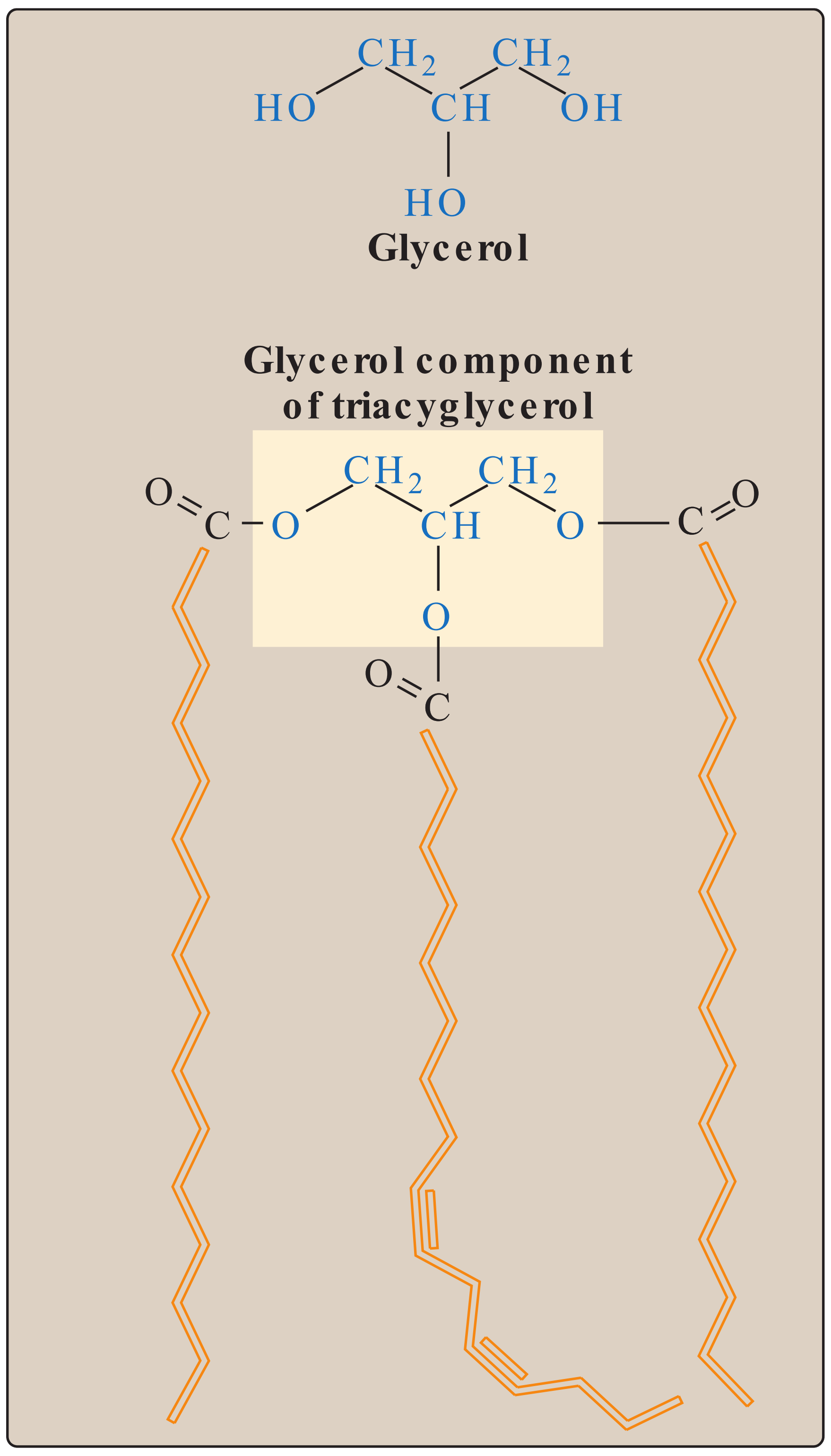

CH2 CH2
HO CH OH
HO
Glycerol
Glycerol component of triacyglycerol
O=C–O CH2 CH2 CH O –C=O
O
O=C

Figure 16.12
A triacylglycerol with an unsaturated fatty acid on carbon 2. Orange denotes the hydrophobic portions of the molecule.

> Humans have carbon 9, 6, 5, and 4 desaturases but lack the ability to introduce double bonds from carbon 10 to the ω end of the chain. This is the basis for the nutritional essentiality of the polyunsaturated acids ω-6 linoleic and ω-3 linolenic.

G. Storage of fatty acids as components of triacylglycerols

Mono-, di-, and triacylglycerols consist of one, two, or three molecules of fatty acid esterified to a molecule of glycerol. Fatty acids are esterified through their carboxyl groups, resulting in a loss of negative charge and formation of "neutral fat." [Note: If a species of acylglycerol is solid at room temperature, it is called a fat, whereas if it is liquid, it is called an oil.]

1. **Structure:** The three fatty acids esterified to a glycerol molecule to form a TAG are usually not of the same type. The fatty acid on carbon 1 is typically saturated, that on carbon 2 is typically unsaturated, and that on carbon 3 can be either. Recall that the presence of the unsaturated fatty acid(s) decrease(s) the T_m of the lipid. An example of a TAG molecule is shown in Figure 16.12.
2. **Storage:** Because TAGs are only slightly soluble in water and cannot form stable micelles by themselves, they coalesce within white adipocytes to form large oily droplets that are nearly anhydrous. These cytosolic lipid droplets are the major energy reserve of the body. [Note: TAGs stored in brown adipocytes serve as a source of heat through nonshivering thermogenesis (see p. 78).]
3. **Synthesis of glycerol 3-phosphate:** Glycerol 3-phosphate is the initial acceptor of fatty acids during TAG synthesis. There are two pathways for its production (Figure 16.13). In both liver (the primary site of TAG synthesis) and adipose tissue, glycerol 3-phosphate can be produced from glucose, using first the reactions of the glycolytic pathway to produce dihydroxyacetone phosphate ([DHAP], see p. 101). DHAP is reduced by glycerol 3-phosphate dehydrogenase to glycerol 3-phosphate. A second pathway found

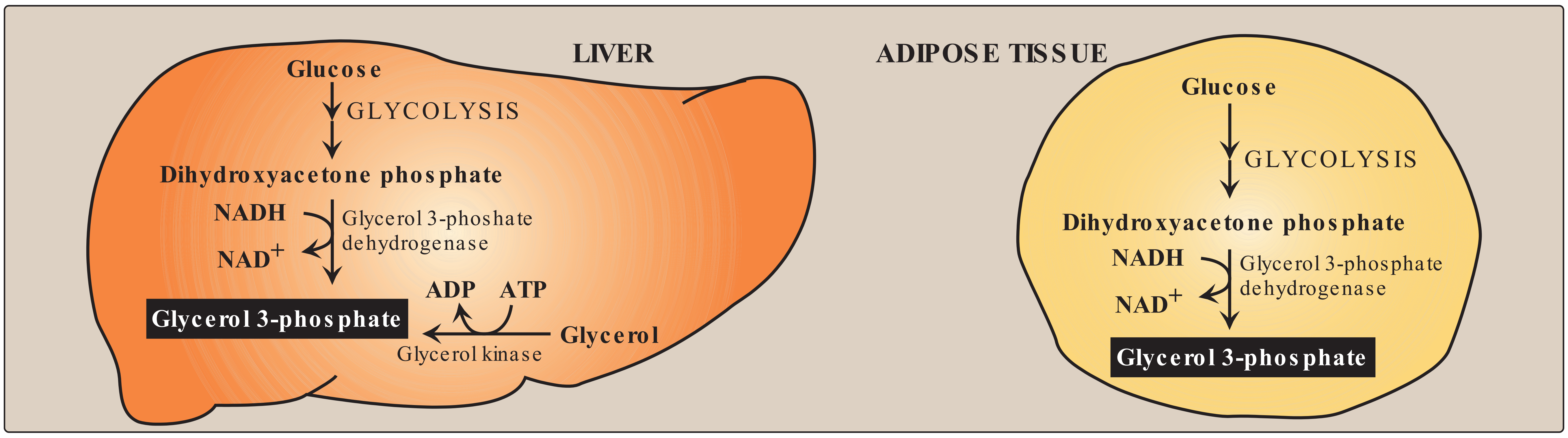

LIVER
Glucose
GLYCOLYSIS
Dihydroxyacetone phosphate
NADH
NAD+
Glycerol 3-phoshate dehydrogenase
ADP ATP
Glycerol 3-phosphate
Glycerol
Glycerol kinase
ADIPOSE TISSUE
Glucose
GLYCOLYSIS
Dihydroxyacetone phosphate
NADH
NAD+
Glycerol 3-phosphate dehydrogenase
Glycerol 3-phosphate

Figure 16.13
Pathways for production of glycerol 3-phosphate in liver and adipose tissue. [Note: Glycerol 3-phosphate can also be generated by glyceroneogenesis.] NAD(H) = nicotinamide adenine dinucleotide; ADP = adenosine diphosphate.

in the liver, but not in adipose tissue, uses glycerol kinase to convert free glycerol to glycerol phosphate (see Figure 16.13). [Note: The glucose transporter in adipocytes (GLUT-4) is insulin dependent (see p. 312). Thus, when plasma glucose (and, therefore, plasma insulin) levels are low, adipocytes have only a limited ability to synthesize glycerol phosphate and cannot produce TAG de novo.]

4. **Activation of a free fatty acid:** A fatty acid must be converted to its activated form (bound to CoA) before it can participate in metabolic processes such as TAG synthesis. This reaction, illustrated in Figure 15.6, is catalyzed by a family of fatty acyl CoA synthetases (thiokinases).

5. **Synthesis of triacylglycerol from glycerol 3-phosphate and fatty acyl coenzyme As:** This pathway involves four reactions, shown in Figure 16.14. These include the sequential addition of two fatty acids from fatty acyl CoAs, the removal of phosphate, and the addition of the third fatty acid.

H. Different fates of triacylglycerol in liver and adipose tissue

In WAT, TAG is stored in a nearly anhydrous form as fat droplets in the cytosol of the cells. It serves as “depot fat,” ready for mobilization when the body requires it for fuel. Little TAG is stored in healthy liver. Instead, most is exported, packaged with other lipids and apolipoproteins to form lipoprotein particles called very-low-density lipoproteins (VLDLs). Nascent VLDLs are secreted directly into the blood where they mature and function to deliver the endogenously derived lipids to the peripheral tissues. [Note: Recall from Chapter 15 that chylomicrons carry dietary (exogenously derived) lipids.] Plasma lipoproteins are discussed in Chapter 18.

IV. MOBILIZATION OF STORED FATS AND OXIDATION OF FATTY ACIDS

Fatty acids stored in WAT, in the form of neutral TAG, serve as the body’s major fuel storage reserve. TAGs provide concentrated stores of metabolic energy because they are highly reduced and largely anhydrous. The yield from the complete oxidation of fatty acids to CO_2 and H_2O is 9 kcal/g fat (as compared to 4 kcal/g protein or carbohydrate, see Figure 27.5 on p. 359).

A. Release of fatty acids from fat

The mobilization of stored fat requires the hydrolytic release of fatty acids and glycerol from their TAG form. This process of lipolysis is achieved by lipases. It is initiated by adipose triglyceride lipase (ATGL), which generates a diacylglycerol that is the preferred substrate for hormone-sensitive lipase (HSL). The monoacylglycerol (MAG) product of HSL is acted upon by MAG lipase.

1. **Regulation of hormone-sensitive lipase:** HSL is active when phosphorylated by PKA, a 3′,5′-cyclic AMP (cAMP)–dependent protein kinase. cAMP is produced in the adipocyte when catecholamines (such as epinephrine) bind to cell membrane β-adrenergic receptors and activate adenylyl cyclase (Figure

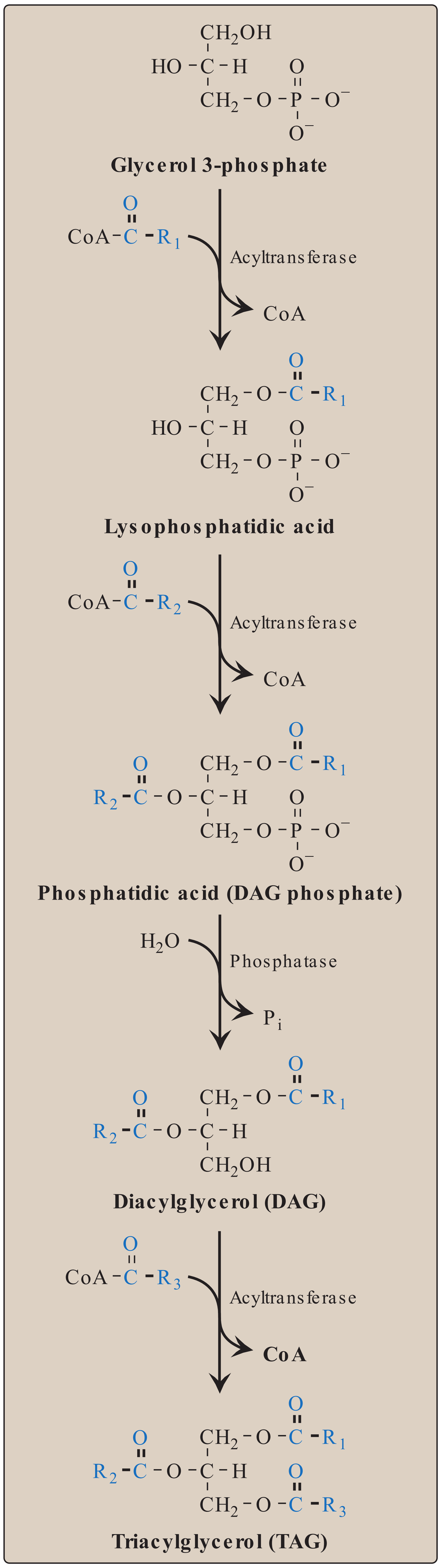


Figure 16.14
Synthesis of TAG. R_1–R_3 = activated fatty acids. CoA = coenzyme A; P_i = inorganic phosphate.

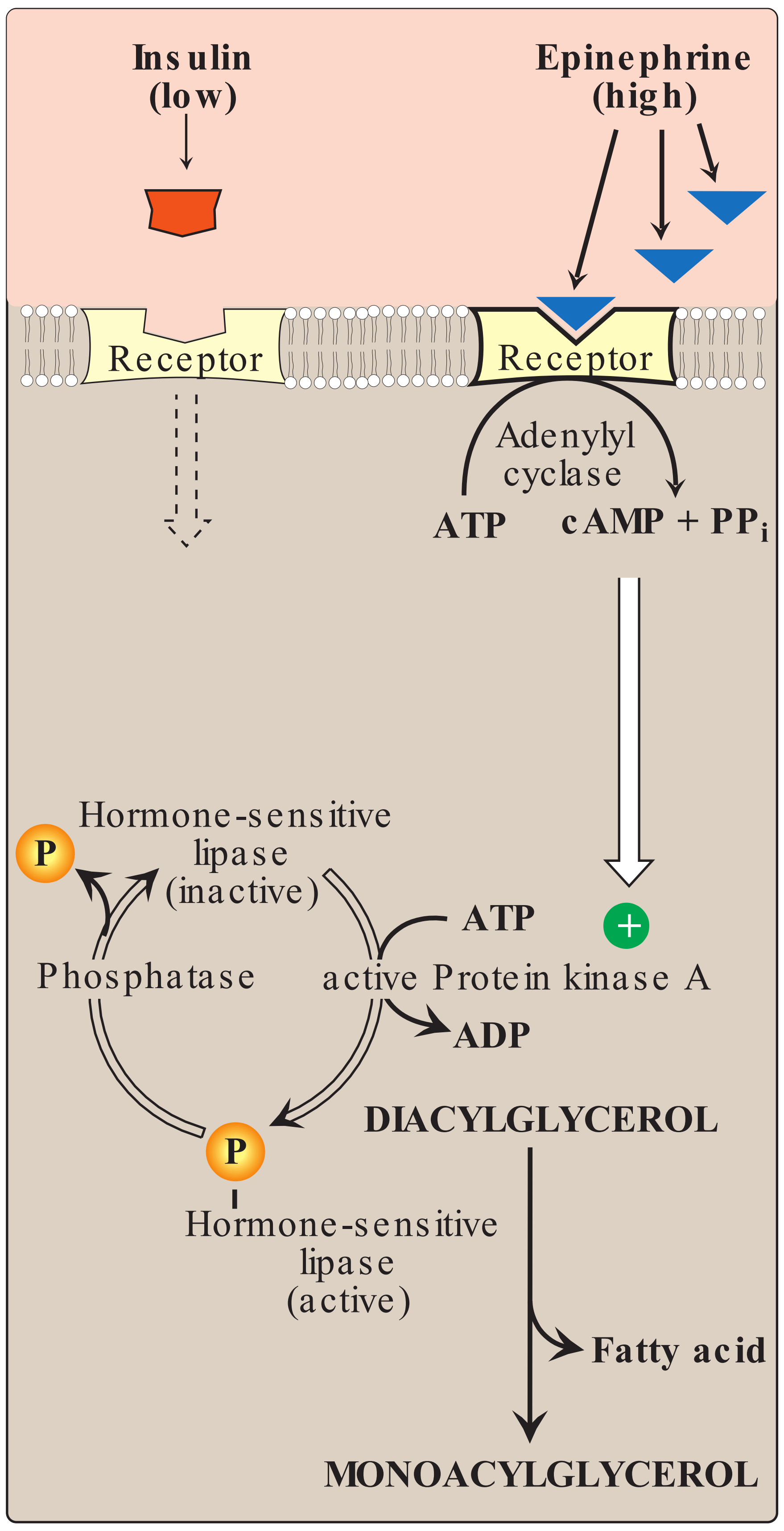


Figure 16.15
Hormonal regulation of fat degradation in the adipocyte. [Note: Triacylglycerol is degraded to diacylglycerol by adipose triglyceride lipase.] cAMP = cyclic adenosine monophosphate; PP$_i$ = pyrophosphate; ADP = adenosine diphosphate; Ⓟ = phosphate.

16.15). The process is similar to that of the activation of glycogen phosphorylase (see Figure 11.9). [Note: Because ACC is inhibited by hormone-directed phosphorylation, when the cAMP-mediated cascade is activated (see Figure 16.8), fatty acid synthesis is turned off and TAG degradation is turned on.] In the presence of high plasma levels of insulin, HSL is dephosphorylated and inactivated. Insulin also suppresses expression of ATGL. [Note: Fat droplets are coated by a protein (perilipin) that limits access of HSL. Phosphorylation of perilipin by PKA allows translocation and binding of HSL to the droplet.]

2. **Fate of glycerol:** The glycerol released during TAG degradation cannot be metabolized by adipocytes because they lack glycerol kinase. Rather, glycerol is transported through the blood to the liver, where it can be phosphorylated. The resulting glycerol 3-phosphate can be used to form TAG in the liver or can be converted to DHAP by reversal of the glycerol 3-phosphate dehydrogenase reaction illustrated in Figure 16.13. DHAP can participate in glycolysis or gluconeogenesis.

3. **Fate of fatty acids:** The free (unesterified) fatty acids move through the cell membrane of the adipocyte and bind to plasma albumin. They are transported to the tissues, enter cells, get activated to their CoA derivatives, and are oxidized for energy in mitochondria. Regardless of their levels, plasma FFAs cannot be used for fuel by red blood cells (RBCs), which have no mitochondria. Brain, too, does not use fatty acids for energy, but the reasons are less clear. [Note: Over 50% of the fatty acids released from adipose TAG are reesterified to glycerol 3-phosphate. WAT does not express glycerol kinase, and the phosphorylated glycerol is produced by glyceroneogenesis, an incomplete version of gluconeogenesis: pyruvate to OAA via pyruvate carboxylase and OAA to phosphoenolpyruvate (PEP) via phosphoenolpyruvate carboxykinase. The PEP is converted (by reactions common to glycolysis and gluconeogenesis) to DHAP, which is reduced to glycerol 3-phosphate. The process reduces plasma FFAs, molecules associated with insulin resistance in type 2 diabetes and obesity (see p. 343).]

B. β-Oxidation of fatty acids

The major pathway for catabolism of fatty acids is a mitochondrial pathway called β-oxidation, in which two-carbon fragments are successively removed from the carboxyl end of the fatty acyl CoA, producing acetyl CoA, NADH, and flavin adenine dinucleotide (FADH$_2$).

1. **Transport of long-chain fatty acids into mitochondria:** After a LCFA enters a cell, it is converted in the cytosol to its CoA derivative by long-chain fatty acyl CoA synthetase (thiokinase), an enzyme of the outer mitochondrial membrane. Because β-oxidation occurs in the mitochondrial matrix, the fatty acid must be transported across the inner mitochondrial membrane that is impermeable to CoA. Therefore, a specialized carrier transports the long-chain acyl group from the cytosol into the mitochondrial matrix. This carrier is carnitine, and this rate-limiting transport process is called the "carnitine shuttle" (Figure 16.16).

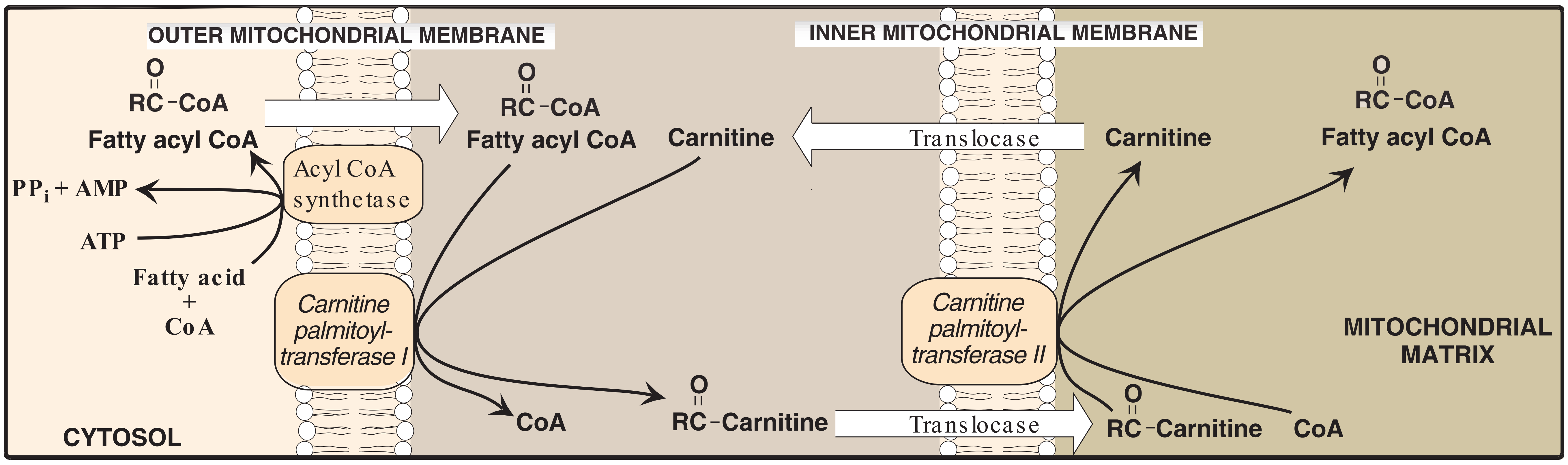

OUTER MITOCHONDRIAL MEMBRANE
INNER MITOCHONDRIAL MEMBRANE
O
RC-CoA
Fatty acyl CoA
PPi + AMP
ATP
Fatty acid + CoA
Acyl CoA synthetase
Carnitine palmitoyl-transferase I
O
RC-CoA
Fatty acyl CoA
Carnitine
Translocase
CoA
O
RC-Carnitine
Translocase
Carnitine palmitoyl-transferase II
Carnitine
O
RC-CoA
Fatty acyl CoA
MITOCHONDRIAL MATRIX
O
RC-Carnitine
CoA
CYTOSOL

Figure 16.16
Carnitine shuttle. The net effect is that a long-chain fatty acyl coenzyme A (CoA) is transported from the outside to the inside of mitochondria. AMP = adenosine monophosphate; PP_i = pyrophosphate.

a. **Steps in translocation:** First, the acyl group is transferred from CoA to carnitine by carnitine palmitoyltransferase I (CPT-I), an enzyme of the outer mitochondrial membrane. [Note: CPT-I is also known as CAT-I for carnitine acyltransferase I.] This reaction forms an acylcarnitine and regenerates free CoA. Second, the acylcarnitine is transported into the mitochondrial matrix in exchange for free carnitine by carnitine–acylcarnitine translocase. Carnitine palmitoyltransferase II (CPT-II, or CAT-II), an enzyme of the inner mitochondrial membrane, catalyzes the transfer of the acyl group from carnitine to CoA in the mitochondrial matrix, thus regenerating free carnitine.

b. **Inhibitor of the carnitine shuttle:** Malonyl CoA inhibits CPT-I, thus preventing the entry of long-chain acyl groups into the mitochondrial matrix. Therefore, when fatty acid synthesis is occurring in the cytosol (as indicated by the presence of malonyl CoA), the newly made palmitate cannot be transferred into mitochondria and degraded. [Note: Muscle, although it does not synthesize fatty acids, contains the mitochondrial isoform of ACC (ACC2), allowing muscle to regulate β-oxidation.] Fatty acid oxidation is also regulated by the acetyl CoA to CoA ratio: as the ratio increases, the CoA-requiring thiolase reaction decreases (Figure 16.17).

c. **Sources of carnitine:** Carnitine can be obtained from the diet, where it is found primarily in meat products. Carnitine can also be synthesized from the amino acids lysine and methionine by an enzymatic pathway found in the liver and kidney but not in skeletal or heart muscle. Therefore, these latter tissues are totally dependent on uptake of carnitine provided by endogenous synthesis or the diet and distributed by the blood. [Note: Skeletal muscle contains about 97% of all carnitine in the body.]

d. **Carnitine deficiencies:** Such deficiencies result in a decreased ability of tissues to use LCFAs as a fuel. Primary carnitine deficiency is caused by defects in a membrane transporter that prevent uptake of carnitine by cardiac and skeletal muscle and kidney. Treatment includes carnitine supplementation. Secondary carnitine deficiency occurs primarily as a result of defects in fatty acid oxidation leading to the accumulation of acylcarnitines that are excreted in the urine, decreasing carnitine availability. Acquired secondary carnitine deficiency can be seen, for example, in patients with liver disease (decreased carnitine synthesis) or those taking the antiseizure drug valproic acid (decreased renal reabsorption). [Note: Defects in mitochondrial oxidation can also be caused by deficiencies in CPT-I and CPT-II. CPT-I deficiency affects the liver, where an inability to use LCFAs for fuel greatly impairs that tissue's ability to synthesize glucose (an endergonic process) during a fast. This can lead to severe hypoglycemia, coma, and death. CPT-II deficiency can affect the liver and cardiac and skeletal muscle. The most common (and least severe) form affects skeletal muscle. It presents as muscle weakness with myoglobinemia following prolonged exercise. Treatment includes avoidance of fasting and adopting a diet high in carbohydrates and low in fat but supplemented with medium-chain TAGs.]

2. **Entry of short- and medium-chain fatty acids into the mitochondria:** Fatty acids shorter than 12 carbons can cross the inner mitochondrial membrane without the aid of carnitine or the CPT system. Once inside the mitochondria, they are activated to their CoA derivatives by matrix enzymes, and are oxidized. [Note: Medium-chain fatty acids are plentiful in human milk. Because their oxidation is not dependent on CPT-I, it is not subject to inhibition by malonyl CoA.]

3. **Reactions of β-oxidation:** The first cycle of β-oxidation is shown in Figure 16.17. It consists of a sequence of four reactions involving the β-carbon (carbon 3) that results in shortening the fatty acid chain by two carbons at the carboxylate end. The steps include an oxidation that produces $FADH_2$, a hydration step, a second oxidation that produces NADH, and a thiolytic cleavage that releases a molecule of acetyl CoA. Each step is catalyzed by enzymes with chain-length specificity. These four steps are repeated for saturated fatty acids of even-numbered carbon chains $(n/2) - 1$ times (where n is the number of carbons), each cycle producing one acetyl CoA plus one NADH and one $FADH_2$. The acetyl CoA can be oxidized or used in hepatic ketogenesis (see below). The reduced coenzymes are oxidized by the electron transport chain. The final thiolytic cleavage produces two acetyl groups. [Note: Acetyl CoA is a positive allosteric effector of pyruvate carboxylase (see p. 119), thus linking fatty acid oxidation and gluconeogenesis.]

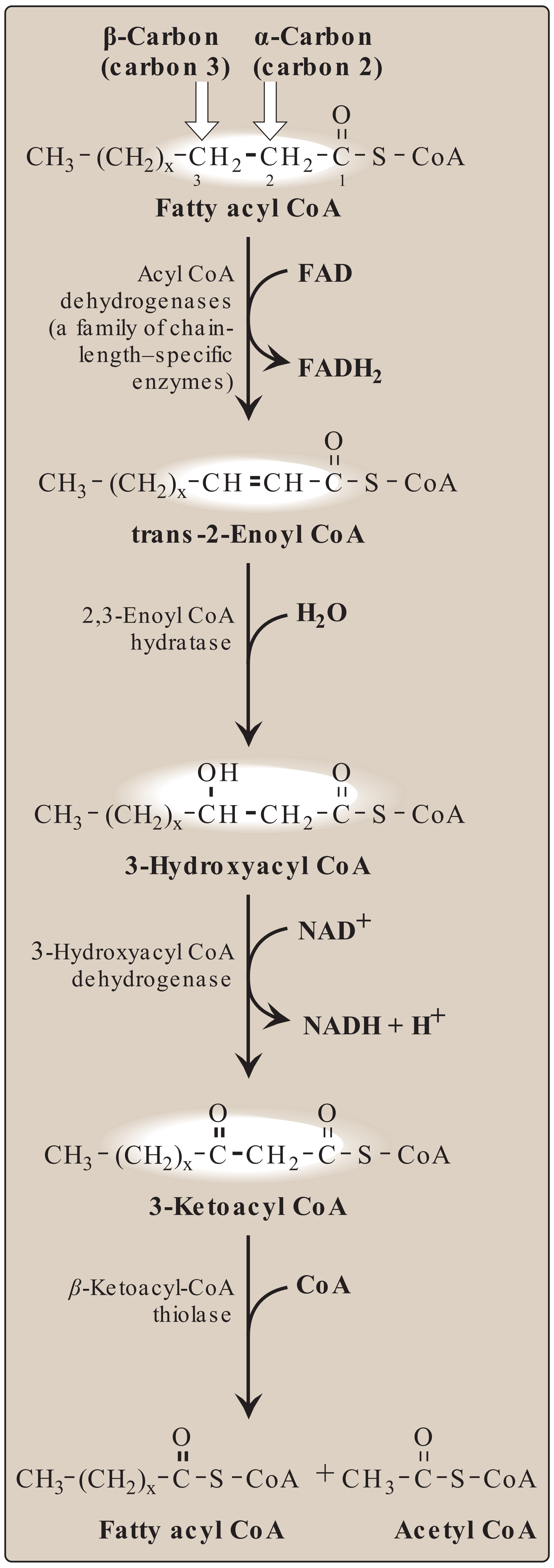
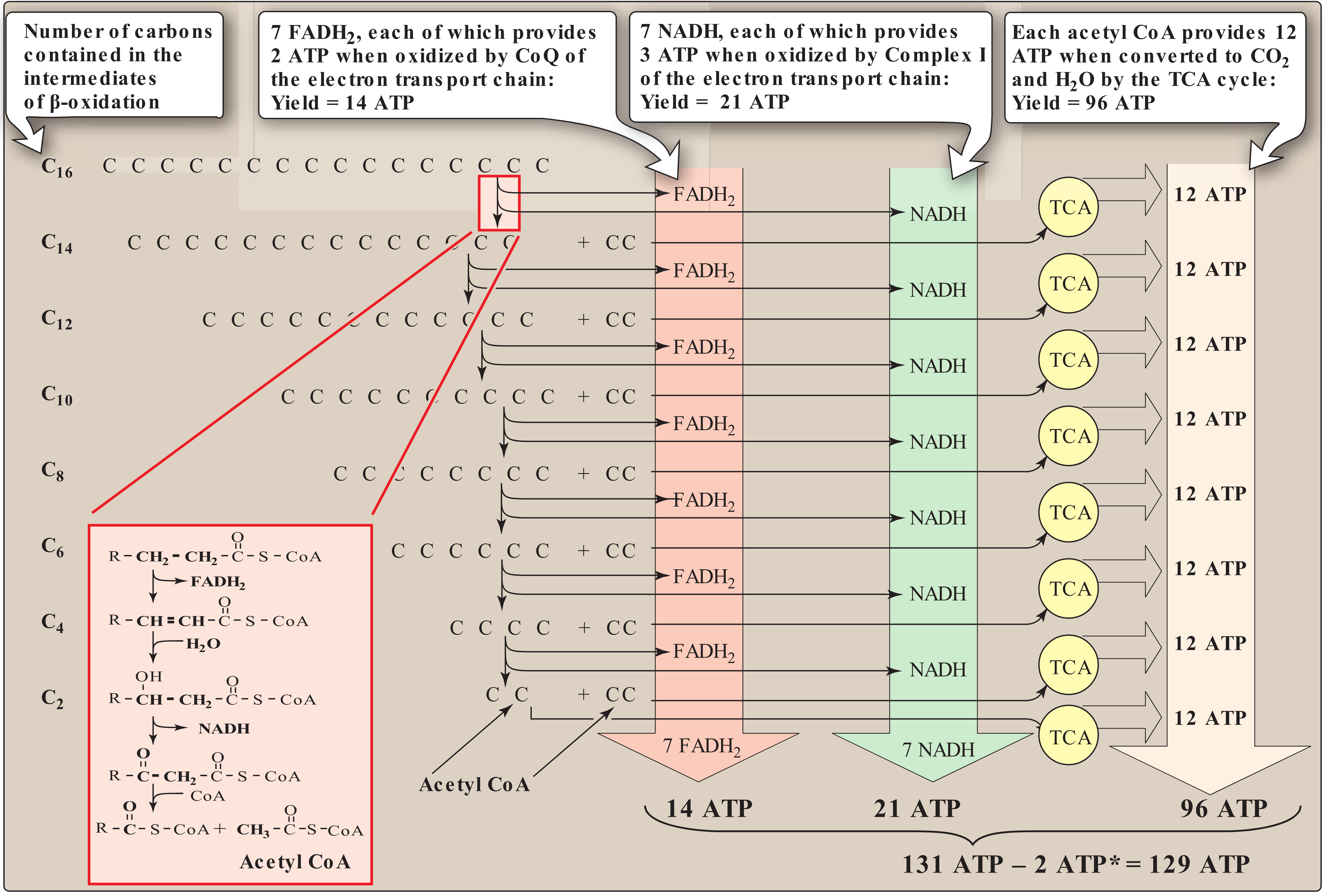

β-Carbon (carbon 3)
α-Carbon (carbon 2)
$CH_3-(CH_2)_x-CH_2-CH_2-C(=O)-S-CoA$
3
2
1
Fatty acyl CoA
Acyl CoA dehydrogenases (a family of chain-length–specific enzymes)
FAD
$FADH_2$
$CH_3-(CH_2)_x-CH=CH-C(=O)-S-CoA$
trans-2-Enoyl CoA
2,3-Enoyl CoA hydratase
H_2O
$CH_3-(CH_2)_x-CH(OH)-CH_2-C(=O)-S-CoA$
3-Hydroxyacyl CoA
3-Hydroxyacyl CoA dehydrogenase
NAD^+
$NADH + H^+$
$CH_3-(CH_2)_x-C(=O)-CH_2-C(=O)-S-CoA$
3-Ketoacyl CoA
β-Ketoacyl-CoA thiolase
CoA
$CH_3-(CH_2)_x-C(=O)-S-CoA + CH_3-C(=O)-S-CoA$
Fatty acyl CoA
Acetyl CoA

Figure 16.17
Enzymes involved in the β-oxidation of fatty acyl coenzyme A (CoA). [Note: 2,3-Enoyl CoA hydratase requires a trans double bond between carbon 2 and carbon 3.] $FAD(H_2)$ = flavin adenine dinucleotide; NAD(H) = nicotinamide adenine dinucleotide.

4. **Energy yield from fatty acid oxidation:** The energy yield from the β-oxidation pathway is high. For example, the oxidation of a molecule of palmitoyl CoA to CO_2 and H_2O produces 8 acetyl CoA, 7 NADH, and 7 $FADH_2$, from which 131 ATP can be generated. However, activation of the fatty acid requires 2 ATP. Therefore, the net yield from palmitate is 129 ATP (Figure 16.18). A comparison of the processes of synthesis and degradation of long-chain saturated fatty acids with an even number of carbon atoms is provided in Figure 16.19.

5. **Medium-chain fatty acyl CoA dehydrogenase deficiency:** In mitochondria, there are four fatty acyl CoA dehydrogenase species, each with distinct but overlapping specificity for either short-, medium-, long-, or VLCFAs. Medium-chain fatty acyl CoA dehy-


Number of carbons contained in the intermediates of β-oxidation

7 $FADH_2$, each of which provides 2 ATP when oxidized by CoQ of the electron transport chain: Yield = 14 ATP

7 NADH, each of which provides 3 ATP when oxidized by Complex I of the electron transport chain: Yield = 21 ATP

Each acetyl CoA provides 12 ATP when converted to CO_2 and H_2O by the TCA cycle: Yield = 96 ATP

C16 C C C C C C C C C C C C C C C C
C14 C C C C C C C C C C C C C C + CC
C12 C C C C C C C C C C C C + CC
C10 C C C C C C C C C C + CC
C8 C C C C C C C C + CC
C6 C C C C C C + CC
C4 C C C C + CC
C2 C C + CC
FADH2
NADH
TCA
12 ATP
7 FADH2
7 NADH
Acetyl CoA
R-CH2-CH2-C(=O)-S-CoA
FADH2
R-CH=CH-C(=O)-S-CoA
H2O
R-CH(OH)-CH2-C(=O)-S-CoA
NADH
R-C(=O)-CH2-C(=O)-S-CoA
CoA
R-C(=O)-S-CoA + CH3-C(=O)-S-CoA
Acetyl CoA
14 ATP
21 ATP
96 ATP

131 ATP – 2 ATP* = 129 ATP

Figure 16.18
Summary of the energy yield from the oxidation of palmitoyl coenzyme A (CoA) (16 carbons). *Activation of palmitate to palmitoyl CoA requires the equivalent of 2 ATP (ATP → AMP + PP_i). $FADH_2$ = flavin adenine dinucleotide; NADH = nicotinamide adenine dinucleotide; TCA = tricarboxylic acid; CoQ = coenzyme Q.

drogenase (MCAD) deficiency, an autosomal-recessive disorder, is one of the most common inborn errors of metabolism and the most common inborn error of fatty acid oxidation, being found in 1:14,000 births worldwide, with a higher incidence in Caucasians of Northern European descent. It results in decreased ability to oxidize fatty acids with six to ten carbons (which accumulate and can be measured in urine), severe hypoglycemia (because the tissues must increase their reliance on glucose), and hypoketonemia (because of decreased production of acetyl CoA). See below. Treatment includes avoidance of fasting. MCAD deficiency has been identified as the cause of some cases originally reported as sudden infant death syndrome or Reye syndrome.

6. **Oxidation of fatty acids with an odd number of carbons:** This process proceeds by the same reaction steps as that of fatty acids with an even number of carbons, until the final three carbons are reached. This compound, propionyl CoA, is metabolized by a three-step pathway (Figure 16.20). [Note: Propionyl CoA is also produced during the metabolism of certain amino acids (see Figure 20.10).]

   a. **Synthesis of D-methylmalonyl coenzyme A:** First, propionyl CoA is carboxylated, forming D-methylmalonyl coenzyme A. The

	SYNTHESIS	DEGRADATION
Greatest flux through pathway	After carbohydrate-rich meal	In starvation
Hormonal state favoring pathway	High insulin/glucagon ratio	Low insulin/glucagon ratio
Major tissue site	Primarily liver	Muscle, liver
Subcellular location	Cytosol	Primarily mitochondria
Carriers of acyl/acetyl groups between mitochondria and cytosol	Citrate (mitochondria to cytosol)	Carnitine (cytosol to mitochondria)
Phosphopantetheine-containing active carriers	Acyl carrier protein domain, coenzyme A	Coenzyme A
Oxidation/reduction coenzymes	NADPH (reduction)	NAD^+, FAD (oxidation)
Two-carbon donor/product	Malonyl CoA: donor of one acetyl group	Acetyl CoA: product of β-oxidation
Activator	Citrate	
Inhibitor	Long-chain fatty acyl CoA (inhibits acetyl CoA carboxylase)	Malonyl CoA (inhibits carnitine palmitoyltransferase-I)
Product of pathway	Palmitate	Acetyl CoA
Repetitive four-step process	Condensation, reduction dehydration, reduction	Dehydrogenation, hydration dehydrogenation, thiolysis

Figure 16.19
Comparison of the synthesis and degradation of long-chain, even-numbered, saturated fatty acids. NADPH = nicotinamide adenine dinucleotide phosphate; NAD = nicotinamide adenine dinucleotide; FAD = flavin adenine dinucleotide; CoA = coenzyme A.

enzyme propionyl CoA carboxylase has an absolute requirement for the coenzyme biotin, as do ACC and most other carboxylases.

b. **Formation of L-methylmalonyl coenzyme A:** Next, the D-isomer is converted to the L-form by the enzyme, methylmalonyl CoA racemase.

c. **Synthesis of succinyl coenzyme A:** Finally, the carbons of L-methylmalonyl CoA are rearranged, forming succinyl CoA, which can enter the tricarboxylic acid (TCA) cycle (see p. 109). [Note: This is the only example of a glucogenic precursor generated from fatty acid oxidation.] The enzyme methylmalonyl CoA mutase requires a coenzyme form of vitamin B_{12} (deoxyadenosylcobalamin). The mutase reaction is one of only two reactions in the body that require vitamin B_{12} (see p. 375). [Note: In patients with vitamin B_{12} deficiency, both propionate and methylmalonate are excreted in the urine. Two types of heritable methylmalonic acidemia and aciduria have been described: one in which the mutase is missing or deficient (or has reduced affinity for the coenzyme), and one in which the patient is unable to convert vitamin B_{12} into its coenzyme form. Either type results in metabolic acidosis and neurologic manifestations.]

7. **Oxidation of unsaturated fatty acids:** The oxidation of unsaturated fatty acids provides less energy than that of saturated fatty acids because unsaturated fatty acids are less highly reduced, and, therefore, fewer reducing equivalents can be produced from these structures. Oxidation of monounsaturated fatty acids, such as 18:1(9) (oleic acid), requires one additional enzyme, 3,2-enoyl CoA isomerase, which converts the 3-cis derivative obtained after three rounds of β-oxidation to the 2-trans derivative required as a substrate by the enoyl CoA hydratase. Oxidation of polyunsaturated fatty acids, such as 18:2(9,12) (linoleic acid), requires an NADPH-dependent 2,4-dienoyl CoA reductase in addition to the isomerase.

8. **β-Oxidation in the peroxisome:** VLCFAs of more than 22 carbons undergo a preliminary β-oxidation in peroxisomes, because peroxisomes are the primary site of the synthetase that activates fatty acids of this length. The shortened fatty acid (linked to carnitine) diffuses to a mitochondrion for further oxidation. In contrast to mitochondrial β-oxidation, the initial dehydrogenation in peroxisomes is catalyzed by a FAD-containing acyl CoA oxidase. The $FADH_2$ produced is oxidized by molecular oxygen, which is reduced to H_2O_2. Therefore, no ATP is generated by this step. The H_2O_2 is reduced to H_2O by catalase (see p. 148). [Note: Genetic defects in the ability either to target matrix proteins to peroxisomes (resulting in Zellweger syndrome, a peroxisomal biogenesis disorder) or to transport VLCFAs across the peroxisomal membrane (resulting in X-linked adrenoleukodystrophy), lead to accumulation of VLCFAs in the blood and tissues.]

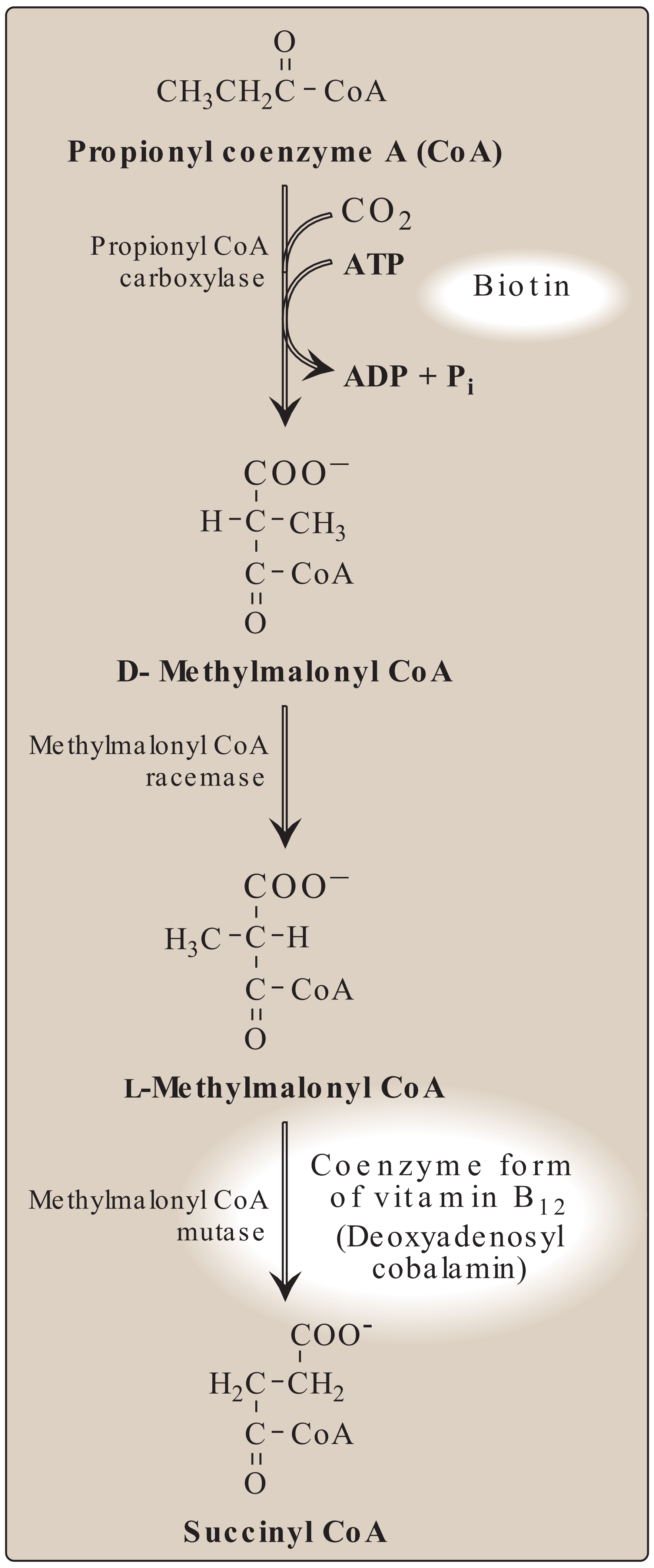


Figure 16.20
Metabolism of propionyl CoA. ADP = adenosine diphosphate; P_i = inorganic phosphate.

C. Peroxisomal a-oxidation of fatty acids

Branched-chain phytanic acid: This product of chlorophyll metabolism is not a substrate for acyl CoA dehydrogenase because of the methyl group on its β-carbon (Figure 16.21). Instead, it is hydroxylated at the α-carbon by phytanoyl CoA α-hydroxylase (PhyH), carbon 1 is released as CO_2, and the product, 19-carbon pristanal, is oxidized to pristanic acid, which is activated to its CoA derivative and undergoes β-oxidation. Refsum disease is a rare, autosomal-recessive disorder caused by a deficiency of peroxisomal PhyH. This results in the accumulation of phytanic acid in the plasma and tissues. The symptoms are primarily neurologic, and the treatment involves dietary restriction to halt disease progression. [Note: ω-Oxidation (at the methyl terminus) also is known and generates dicarboxylic acids. Normally a minor pathway of the ER, its upregulation is seen with conditions such as MCAD deficiency that limit fatty acid β-oxidation.]

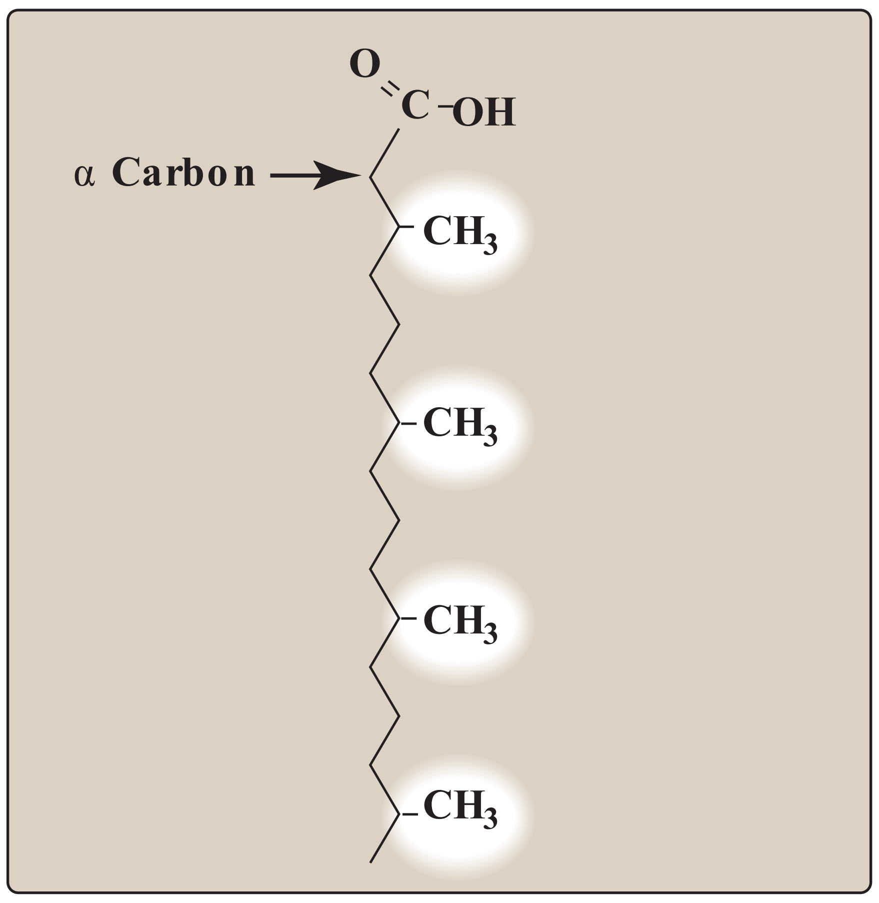


Figure 16.21
Phytanic acid, a branched-chain fatty acid.

V. KETONE BODIES: AN ALTERNATE FUEL FOR CELLS

Liver mitochondria have the capacity to convert acetyl CoA derived from fatty acid oxidation into ketone bodies. The compounds categorized as ketone bodies are acetoacetate, 3-hydroxybutyrate (also called β-hydroxybutyrate), and acetone (a nonmetabolized side product, Figure 16.22). [Note: The two functional ketone bodies are actually organic acids.] Acetoacetate and 3-hydroxybutyrate are transported in the blood to the peripheral tissues. There they can be reconverted to acetyl CoA, which can be oxidized by the TCA cycle. Ketone bodies are important sources of energy for the peripheral tissues because 1) they are soluble in aqueous solution and, therefore, do not need to be incorporated into lipoproteins or carried by albumin as do the other lipids; 2) they are produced in the liver during periods when the amount of acetyl CoA present exceeds the oxidative capacity of the liver; and 3) they are used in proportion to their concentration in the blood by extrahepatic tissues, such as the skeletal and cardiac muscle, intestinal mucosa, and renal cortex. Even the brain can use ketone bodies to help meet its energy needs if the blood levels rise sufficiently. Thus, ketone bodies spare glucose, which is particularly important during prolonged periods of fasting (see p. 332). [Note: Disorders of fatty acid oxidation present with the general picture of hypoketosis (due to decreased availability of acetyl CoA) and hypoglycemia (due to increased reliance on glucose for energy.]

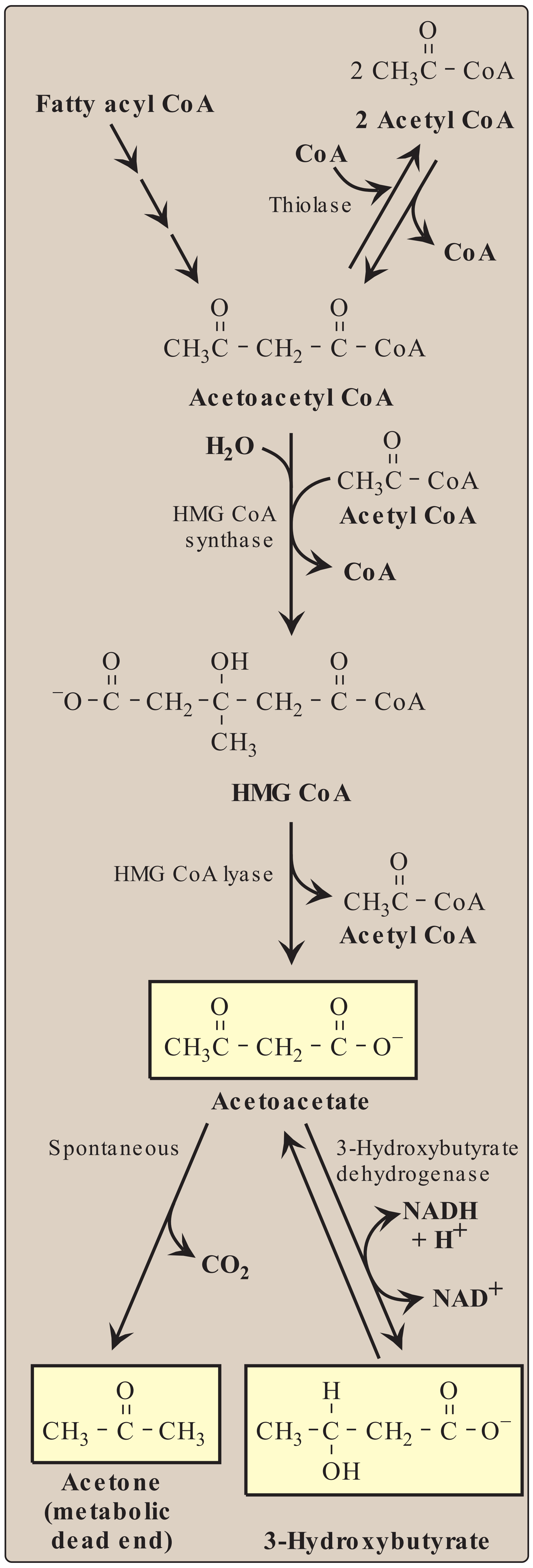


Figure 16.22
Synthesis of ketone bodies. [Note: The release of CoA in ketogenesis supports continued fatty acid oxidation.] CoA = coenzyme A; HMG = hydroxymethylglutarate; NAD(H) = nicotinamide adenine dinucleotide.

A. Synthesis of ketone bodies by the liver: ketogenesis

During a fast, the liver is flooded with fatty acids mobilized from adipose tissue. The resulting elevated hepatic acetyl CoA produced by fatty acid oxidation inhibits pyruvate dehydrogenase (see p. 111), and activates pyruvate carboxylase (see p. 119). The OAA produced is used by the liver for gluconeogenesis rather than for the TCA cycle. Therefore, acetyl CoA is channeled into ketone body synthesis. Additionally, fatty acid oxidation decreases the NAD^+ to NADH ratio, and the rise in NADH shifts OAA to malate (see p. 113). This also pushes acetyl CoA into ketogenesis (Figure 16.24).] [Note: Acetyl CoA for ketogenesis is also generated by the catabolism of ketogenic amino acids (see p. 262).]

1. **Synthesis of 3-hydroxy-3-methylglutaryl coenzyme A:** The first step, formation of acetoacetyl CoA, occurs by reversal of the thiolase reaction of fatty acid oxidation (see Figure 16.17). Mitochondrial 3-hydroxy-3-methylglutaryl (HMG) CoA synthase combines a third molecule of acetyl CoA with acetoacetyl CoA to produce HMG CoA. HMG CoA synthase is the rate-limiting step in the synthesis of ketone bodies and is present in significant quantities only in the liver. [Note: HMG CoA is also an intermediate in cytosolic cholesterol synthesis (see p. 220). The two pathways are separated by location in, and conditions of, the cell.]

2. **Synthesis of the ketone bodies:** HMG CoA is cleaved by HMG CoA lyase to produce acetoacetate and acetyl CoA, as shown in Figure 16.22. Acetoacetate can be reduced to form 3-hydroxybutyrate with NADH as the hydrogen donor. Acetoacetate can also spontaneously decarboxylate in the blood to form acetone, a volatile, biologically nonmetabolized compound that can be released in the breath. The equilibrium between acetoacetate and 3-hydroxybutyrate is determined by the NAD^+/NADH ratio. Because this ratio is low during fatty acid oxidation, 3-hydroxybutyrate synthesis is favored. [Note: The generation of free CoA during ketogenesis allows fatty acid oxidation to continue.]

B. Use of ketone bodies by the peripheral tissues: ketolysis

Although the liver constantly synthesizes low levels of ketone bodies, their production becomes much more significant during fasting when ketone bodies are needed to provide energy to the periph-

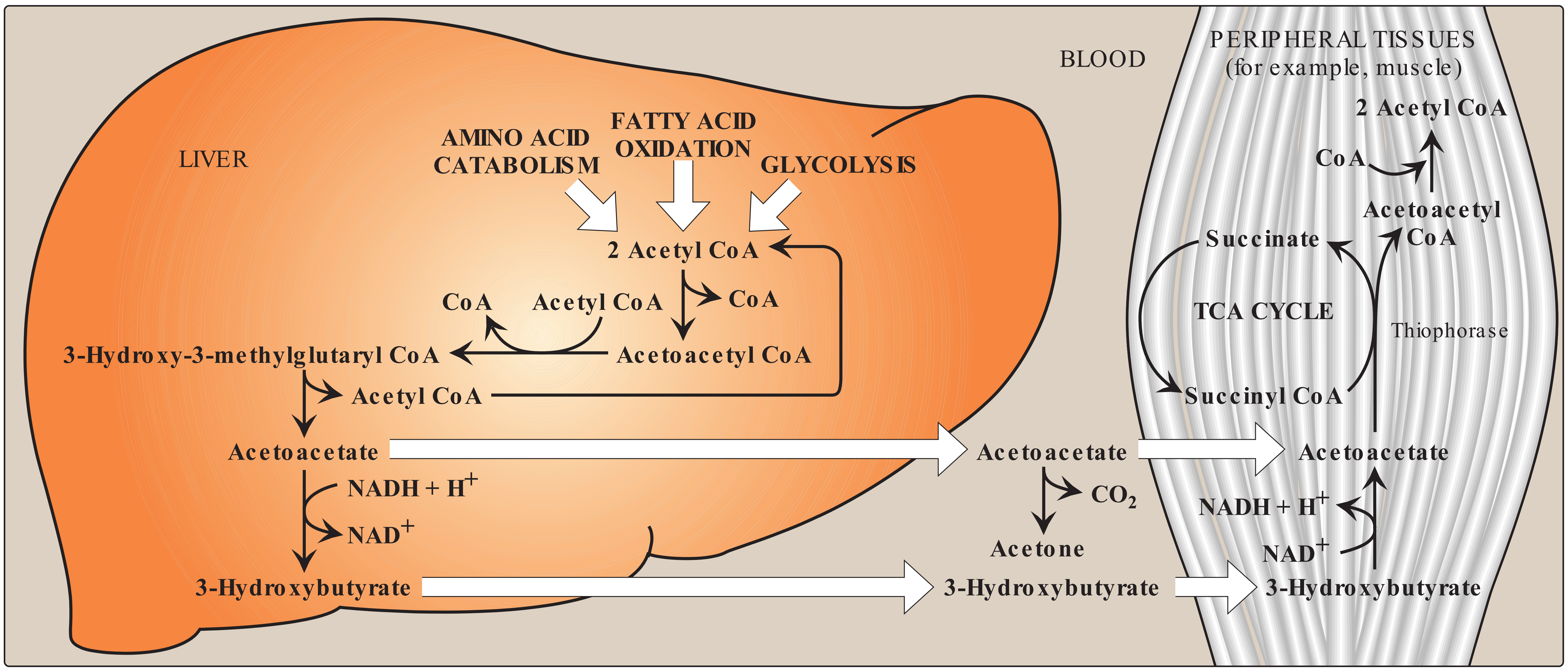

LIVER
AMINO ACID CATABOLISM
FATTY ACID OXIDATION
GLYCOLYSIS
2 Acetyl CoA
CoA
Acetyl CoA
CoA
3-Hydroxy-3-methylglutaryl CoA
Acetoacetyl CoA
Acetyl CoA
Acetoacetate
NADH + H$^+$
NAD$^+$
3-Hydroxybutyrate
BLOOD
Acetoacetate
CO$_2$
Acetone
3-Hydroxybutyrate
PERIPHERAL TISSUES (for example, muscle)
2 Acetyl CoA
CoA
Acetoacetyl CoA
Succinate
TCA CYCLE
Thiophorase
Succinyl CoA
Acetoacetate
NADH + H$^+$
NAD$^+$
3-Hydroxybutyrate

Figure 16.23
Ketone body synthesis in the liver and use in peripheral tissues. Liver and red blood cells cannot use ketone bodies. [Note: Thiophorase is also known as succinyl CoA:acetoacetate CoA transferase.] CoA = coenzyme A; NAD(H) = nicotinamide adenine dinucleotide; TCA = tricarboxylic acid.

eral tissues. 3-Hydroxybutyrate is oxidized to acetoacetate by 3-hydroxybutyrate dehydrogenase, producing NADH (Figure 16.23). Acetoacetate is then provided with a CoA molecule taken from succinyl CoA by succinyl CoA:acetoacetate CoA transferase (thiophorase). This reaction is reversible, but the product, acetoacetyl CoA, is actively removed by its conversion to two acetyl CoAs. This pulls the reaction forward. Extrahepatic tissues, including the brain but excluding cells lacking mitochondria (for example, RBCs), efficiently oxidize acetoacetate and 3-hydroxybutyrate in this manner. In contrast, although the liver actively produces ketone bodies, it lacks thiophorase and, therefore, is unable to use ketone bodies as fuel.

C. Excessive production of ketone bodies in diabetes mellitus

When the rate of formation of ketone bodies is greater than the rate of their use, their levels begin to rise in the blood (ketonemia) and, eventually, in the urine (ketonuria). This is seen most often in cases of uncontrolled type 1 diabetes mellitus. In diabetic individuals with severe ketosis, urinary excretion of the ketone bodies may be as high as 5,000 mg/24 hr, and the blood concentration may reach 90 mg/dl (versus less than 3 mg/dl in normal individuals). A frequent symptom of diabetic ketoacidosis (DKA) is a fruity odor on the breath, which results from increased production of acetone. An elevation of the ketone body concentration in the blood results in acidemia. [Note: The carboxyl group of a ketone body has a pK$_a$ of about 4. Therefore, each ketone body loses a proton (H$^+$) as it circulates in the blood, which lowers the pH. Also, in DKA, urinary loss of glucose and ketone bodies results in dehydration. Therefore, the increased number of H$^+$ circulating in a decreased volume of plasma can cause severe acidosis (ketoacidosis).] Ketoacidosis may also be seen in cases of prolonged fasting (see p. 330) and excessive ethanol consumption (see p. 318).

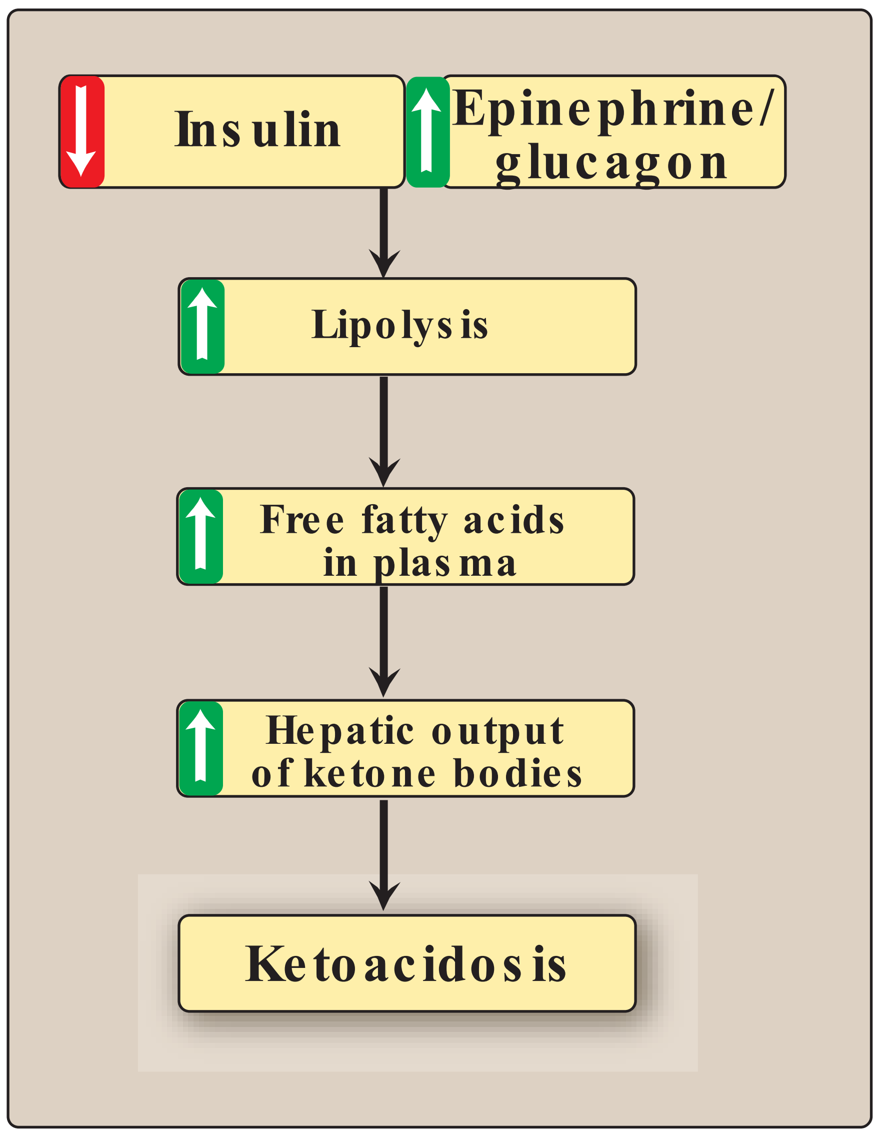

Insulin
Epinephrine/ glucagon
Lipolysis
Free fatty acids in plasma
Hepatic output of ketone bodies
Ketoacidosis

Figure 16.24
Mechanism of diabetic ketoacidosis seen in type 1 diabetes.

VI. CHAPTER SUMMARY

Generally a linear hydrocarbon chain with a terminal carboxyl group, a fatty acid can be **saturated** or **unsaturated**. Two fatty acids are dietary essentials: **linoleic** and **a-linolenic acids**. Fatty acids are synthesized in the cytosol of **liver** following a meal containing excess carbohydrate and protein. Carbons used to synthesize fatty acids are provided by **acetyl coenzyme A (CoA)**, energy by **ATP**, and reducing equivalents by **nicotinamide adenine dinucleotide phosphate** ([**NADPH**]**;** Figure 16.25) provided by the **pentose phosphate pathway** and malic enzyme. **Citrate** carries two-carbon acetyl units from the mitochondrial matrix to the cytosol. The regulated step in fatty acid synthesis is catalyzed by **biotin**-requiring acetyl CoA carboxylase (ACC). **Citrate** allosterically **activates** ACC and **long-chain fatty acyl CoAs inhibit it**. ACC can also be activated by **insulin** and inactivated by **adenosine monophosphate–activated protein kinase** (AMPK) in response to **epinephrine**, **glucagon**, or a **rise in AMP**. The remaining steps in fatty acid synthesis are catalyzed by the multifunctional enzyme, fatty acid synthase, which produces **palmitoyl CoA** by adding two-carbon units from malonyl CoA to a series of acyl acceptors. Fatty acids can be elongated and desaturated in the endoplasmic reticulum (ER). When fatty acids are required for energy, adipocyte hormone-sensitive lipase (**activated** by **epinephrine**, and **inhibited** by **insulin**), along with other lipases, degrades stored triacylglycerol (TAG). The fatty acid products are carried by **serum albumin** to the liver and peripheral tissues, where oxidation of the fatty acids provides energy. The **glycerol** backbone of the degraded TAG is carried by the blood to the **liver**, where it serves as an important **gluconeogenic precursor**. Fatty acid degradation (**β-oxidation**) occurs in **mitochondria**. The **carnitine shuttle** is required to transport **long-chain fatty acids** from the cytosol to the mitochondrial matrix. A translocase and the enzymes carnitine palmitoyltransferases (CPT) I and II are required. CPT-I is **inhibited** by **malonyl CoA**, thereby preventing simultaneous synthesis and degradation of fatty acids. In the mitochondria, fatty acids are oxidized, producing **acetyl CoA**, **nicotinamide adenine dinucleotide** (**NADH**), and **flavin adenine dinucleotide** (**$FADH_2$**). The first step in the β-oxidation pathway is catalyzed by one of four acyl CoA dehydrogenases, each with chain-length specificity. Medium-chain fatty acyl CoA dehydrogenase (MCAD) **deficiency** causes a decrease in fatty acid oxidation (process stops once a medium chain fatty acid is produced), resulting in hypoketonemia and severe hypoglycemia. Oxidation of fatty acids with an odd number of carbons proceeds two carbons at a time (producing acetyl CoA) until three-carbon **propionyl CoA** remains. This compound is carboxylated to **methylmalonyl CoA** (by **biotin-requiring** propionyl CoA carboxylase), which is then converted to **succinyl CoA** (a gluconeogenic precursor) by **vitamin B_{12}**-requiring methylmalonyl CoA mutase. A genetic error in the mutase or vitamin B_{12} deficiency causes **methylmalonic acidemia** and **aciduria**. β-Oxidation of **very-long-chain fatty acids** and α-oxidation of branched-chain fatty acids occur in the peroxisome. ω-Oxidation, a minor pathway, occurs in the ER. Liver mitochondria can convert acetyl CoA derived from fatty acid oxidation into the ketone bodies **acetoacetate** and **3-hydroxybutyrate**. Peripheral tissues possessing mitochondria can oxidize 3-hydroxybutyrate to acetoacetate, which can be reconverted to acetyl CoA, thereby producing energy for the cell. Unlike fatty acids, ketone bodies are utilized by the brain and, therefore, are important fuels during a fast. Because the liver lacks the ability to degrade ketone bodies, it synthesizes them specifically for the peripheral tissues. **Ketoacidosis** occurs when the rate of ketone body formation is greater than the rate of use, as is seen in cases of uncontrolled **type 1 diabetes mellitus**.

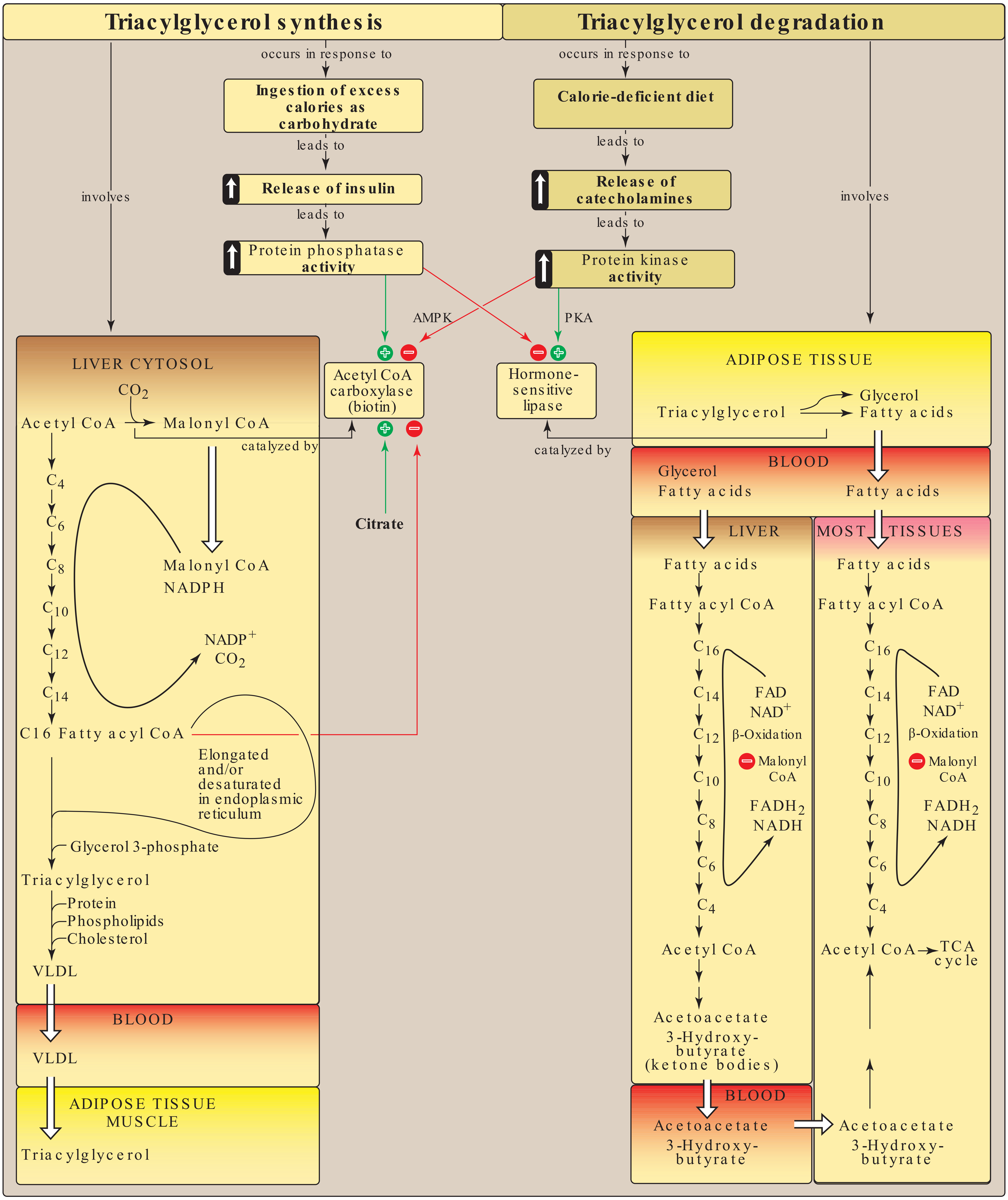

Triacylglycerol synthesis
Triacylglycerol degradation
occurs in response to
Ingestion of excess calories as carbohydrate
leads to
Release of insulin
leads to
Protein phosphatase activity
occurs in response to
Calorie-deficient diet
leads to
Release of catecholamines
leads to
Protein kinase activity
involves
involves
AMPK
PKA
Acetyl CoA carboxylase (biotin)
Hormone-sensitive lipase
catalyzed by
catalyzed by
Citrate
LIVER CYTOSOL
CO2
Acetyl CoA
Malonyl CoA
C4
C6
C8
C10
C12
C14
Malonyl CoA
NADPH
NADP+
CO2
C16 Fatty acyl CoA
Elongated and/or desaturated in endoplasmic reticulum
Glycerol 3-phosphate
Triacylglycerol
Protein
Phospholipids
Cholesterol
VLDL
BLOOD
VLDL
ADIPOSE TISSUE
MUSCLE
Triacylglycerol
ADIPOSE TISSUE
Triacylglycerol
Glycerol
Fatty acids
BLOOD
Glycerol
Fatty acids
Fatty acids
LIVER
Fatty acids
Fatty acyl CoA
C16
C14
C12
C10
C8
C6
C4
FAD
NAD+
β-Oxidation
Malonyl CoA
FADH2
NADH
Acetyl CoA
Acetoacetate
3-Hydroxybutyrate (ketone bodies)
BLOOD
Acetoacetate
3-Hydroxybutyrate
MOST TISSUES
Fatty acids
Fatty acyl CoA
C16
C14
C12
C10
C8
C6
C4
FAD
NAD+
β-Oxidation
Malonyl CoA
FADH2
NADH
Acetyl CoA
TCA cycle
Acetoacetate
3-Hydroxybutyrate

Figure 16.25

Key concept map for fatty acid and triacylglycerol metabolism. AMPK = adenosine monophosphate–activated protein kinase; PKA = protein kinase A; CoA = coenzyme A; NADP(H) = nicotinamide adenine dinucleotide phosphate; FAD(H_2) = flavin adenine dinucleotide; NAD(H) = nicotinamide adenine dinucleotide; TCA = tricarboxylic acid; VLDL = very-low-density lipoprotein.

Phospholipid, Glycosphingolipid, and Eicosanoid Metabolism

17

I. OVERVIEW OF PHOSPHOLIPIDS

Phospholipids are polar, ionic compounds composed of an alcohol that is attached by a phosphodiester bond to either diacylglycerol (DAG) or sphingosine. Like fatty acids, phospholipids are amphipathic in nature. That is, each has a hydrophilic head, which is the phosphate group plus whatever alcohol is attached to it (for example, serine, ethanolamine, and choline, highlighted in blue in Figure 17.1A), and a long, hydrophobic tail containing fatty acids or fatty acid–derived hydrocarbons (shown in orange in Figure 17.1A). Phospholipids are the predominant lipids of cell membranes. In membranes, the hydrophobic portion of a phospholipid molecule is associated with the nonpolar portions of other membrane constituents, such as glycolipids, proteins, and cholesterol. The hydrophilic (polar) head of the phospholipid extends outward, interacting with the intracellular or extracellular aqueous environment (see Figure 17.1A). Membrane phospholipids also function as a reservoir for intracellular messengers, and, for some proteins, phospholipids serve as anchors to cell membranes. Nonmembrane phospholipids serve additional functions in the body, for example, as components of lung surfactant and essential components of bile, where their detergent properties aid in the solubilization of cholesterol.

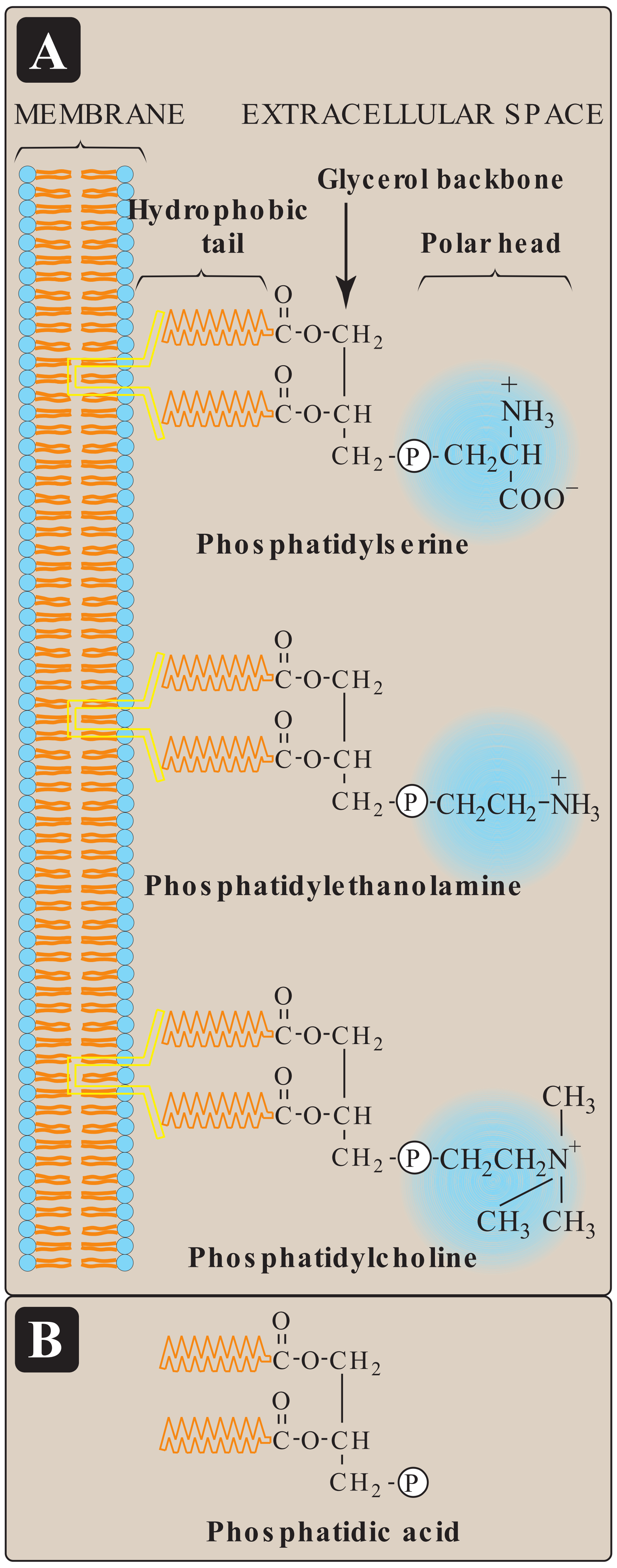


Figure 17.1
A. Structures of some glycerophospholipids. B. Phosphatidic acid. Ⓟ = phosphate (an anion).

II. STRUCTURE OF PHOSPHOLIPIDS

There are two classes of phospholipids: those that have glycerol (from glucose) as a backbone and those that have sphingosine (from serine and palmitate). Both classes are found as structural components of membranes, and both play a role in the generation of lipid-signaling molecules.

A. Glycerophospholipids

Phospholipids that contain glycerol are called glycerophospholipids (or phosphoglycerides). Glycerophospholipids constitute the major class of phospholipids and are the predominant lipids in membranes. All contain (or are derivatives of) phosphatidic acid (PA), which is DAG with a phosphate group on carbon 3 (Figure 17.1B). PA is the simplest phosphoglyceride and is the precursor of the other members of this group.

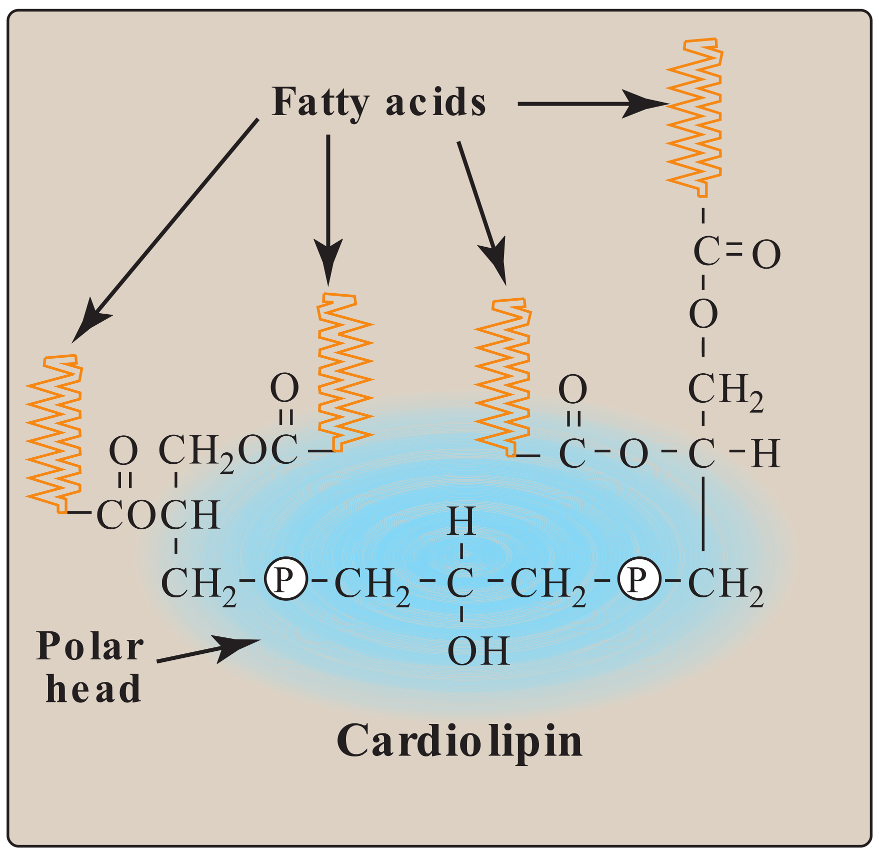


Figure 17.2
Structure of cardiolipin (diphosphatidylglycerol). Ⓟ = phosphate.

1. **Glycerophospholipids from phosphatidic acid and an alcohol:** The phosphate group on PA can be esterified to another compound containing an alcohol group (see Figure 17.1). For example:

Serine + PA → phosphatidylserine (PS)
Ethanolamine + PA → phosphatidylethanolamine (PE) (cephalin)
Choline + PA → phosphatidylcholine (PC) (lecithin)
Inositol + PA → phosphatidylinositol (PI)
Glycerol + PA → phosphatidylglycerol (PG)

2. **Cardiolipin:** Two molecules of PA esterified through their phosphate groups to an additional molecule of glycerol is called cardiolipin, or diphosphatidylglycerol (Figure 17.2). Cardiolipin is found in membranes in bacteria and eukaryotes. In eukaryotes, cardiolipin is virtually exclusive to the inner mitochondrial membrane, where it maintains the structure and function of certain respiratory complexes of the electron transport chain. [Note: Cardiolipin is antigenic and is recognized by antibodies raised against Treponema pallidum, the bacterium that causes syphilis.]

3. **Plasmalogens:** When the fatty acid at carbon 1 of a glycerophospholipid is replaced by an unsaturated alkyl group attached by an ether (rather than by an ester) linkage to the core glycerol molecule, an ether phosphoglyceride known as a plasmalogen is produced. For example, phosphatidalethanolamine, which is abundant in nerve tissue (Figure 17.3A), is the plasmalogen that is similar in structure to phosphatidylethanolamine. Phosphatidalcholine (abundant in heart muscle) is the other quantitatively significant ether lipid in mammals. [Note: Plasmalogens use "al" rather than "yl" in their names.]

4. **Platelet-activating factor:** A second example of an ether glycerophospholipid is platelet-activating factor (PAF), which has a saturated alkyl group in an ether link to carbon 1 and an acetyl residue (rather than a fatty acid) at carbon 2 of the glycerol backbone (Figure 17.3B). PAF is synthesized and released by a variety of cell types. It binds to surface receptors, triggering potent thrombotic and acute inflammatory events. For example, PAF activates inflammatory cells and mediates hypersensitivity, acute inflammatory, and anaphylactic reactions. It causes platelets to aggregate and activate, and neutrophils and alveolar macrophages to generate superoxide radicals to kill bacteria (see p. 148). It also lowers blood pressure. [Note: PAF is one of the most potent bioactive molecules known, causing effects at concentrations as low as 10^{-11} mol/l.]

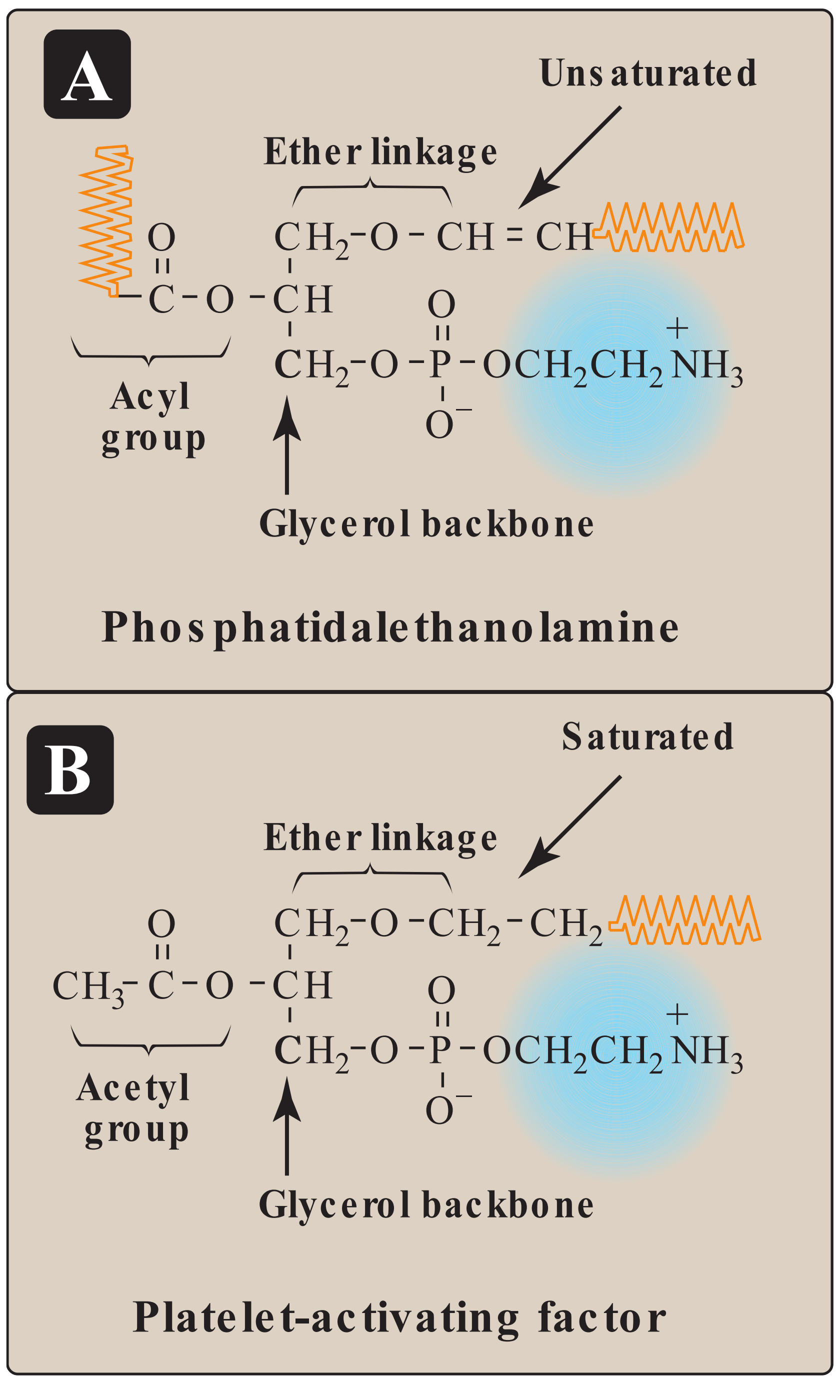


Figure 17.3
The ether glycerophospholipids. A. The plasmalogen phosphatidalethanolamine. B. Platelet-activating factor. (〰 is a long, hydrophobic hydrocarbon chain.)

B. Sphingophospholipids: sphingomyelin

The backbone of sphingomyelin is the amino alcohol sphingosine, rather than glycerol (Figure 17.4). A long-chain fatty acid is attached to the amino group of sphingosine through an amide linkage, producing a ceramide, which can also serve as a precursor of glycolipids (see p. 209). The alcohol group at carbon 1 of sphingosine is esterified to phosphorylcholine, producing sphingomyelin, the only significant sphingophospholipid in humans. Sphingomyelin is an important constituent of the myelin sheath of nerve fibers. [Note: The myelin sheath is a layered, membranous structure that insulates and protects neuronal fibers of the central nervous system (CNS).]

III. PHOSPHOLIPID SYNTHESIS

Glycerophospholipid synthesis involves either the donation of PA from cytidine diphosphate (CDP)-diacylglycerol to an alcohol or the donation of the phosphomonoester of the alcohol from CDP-alcohol to 1,2-diacylglycerol (Figure 17.5). In both cases, the CDP-bound structure is considered an "activated intermediate," and cytidine monophosphate (CMP) is released as a side product of glycerophospholipid synthesis. A key concept in phosphoglyceride synthesis, therefore, is activation, either of DAG or the alcohol to be added, by linkage with CDP. [Note: This is similar in principle to the activation of sugars by their attachment to uridine diphosphate (UDP) (see p. 126).] The fatty acids esterified to the glycerol alcohol groups can vary widely, contributing to the heterogeneity of this group of compounds, with saturated fatty acids typically found at carbon 1 and unsaturated ones at carbon 2. Most phospholipids are synthesized in the smooth endoplasmic reticulum (ER). From there, they are transported to the Golgi apparatus and then to membranes of organelles or the plasma membrane or are secreted from the cell by exocytosis. [Note: Ether lipid synthesis from dihydroxyacetone phosphate occurs in peroxisomes.]

A. Phosphatidic acid

PA is the precursor of many other phosphoglycerides. The steps in its synthesis from glycerol phosphate and two fatty acyl coenzyme A (CoA) molecules were illustrated in Figure 16.14, p. 189, in which PA is shown as a precursor of triacylglycerol.

> Essentially all cells except mature erythrocytes can synthesize phospholipids, whereas triacylglycerol synthesis occurs essentially only in liver, adipose tissue, lactating mammary glands, and intestinal mucosal cells.

B. Phosphatidylcholine and phosphatidylethanolamine

PC and PE are the most abundant phospholipids in most eukaryotic cells. The primary route of their synthesis uses choline and ethanolamine obtained either from the diet or from the turnover of the body's phospholipids. [Note: In the liver, PC also can be synthesized from PS and PE (see below).]

1. **Synthesis from preexisting choline and ethanolamine:** These synthetic pathways involve the phosphorylation of choline or ethanolamine by kinases, followed by conversion to the activated form, CDP-choline or CDP-ethanolamine. Finally, choline-phosphate or ethanolamine-phosphate is transferred from the nucleotide (leaving CMP) to a molecule of DAG (see Figure 17.5).

   a. **Significance of choline reutilization:** The reutilization of choline is important because, whereas humans can synthesize choline de novo, the amount made is insufficient for our needs. Thus, choline is an essential dietary nutrient with an Adequate

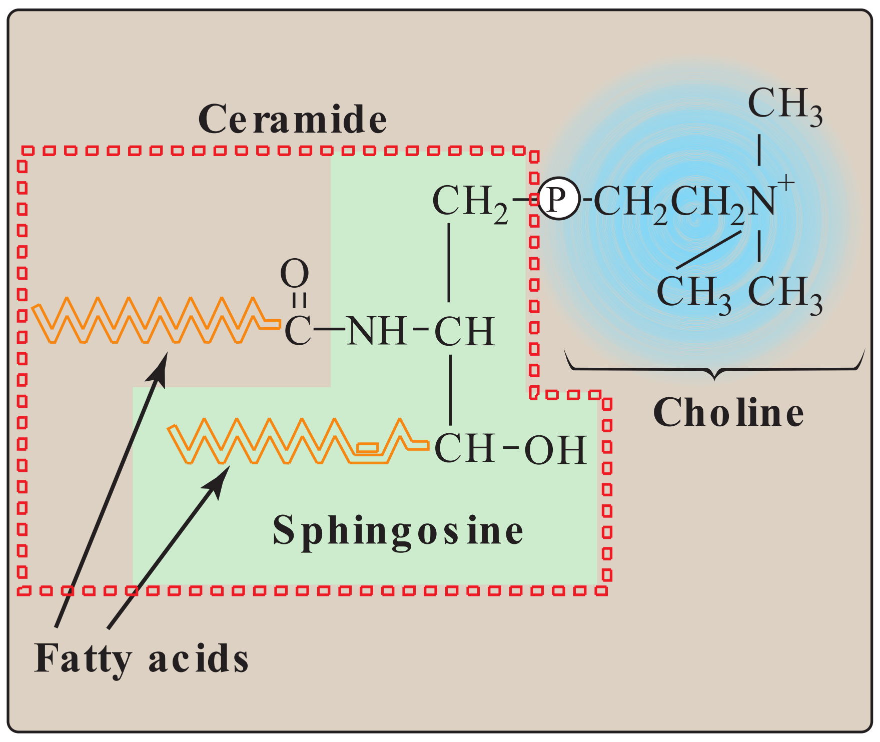


Figure 17.4
Structure of sphingomyelin, showing sphingosine (in green box) and ceramide components (in dashed box). Ⓟ = phosphate.

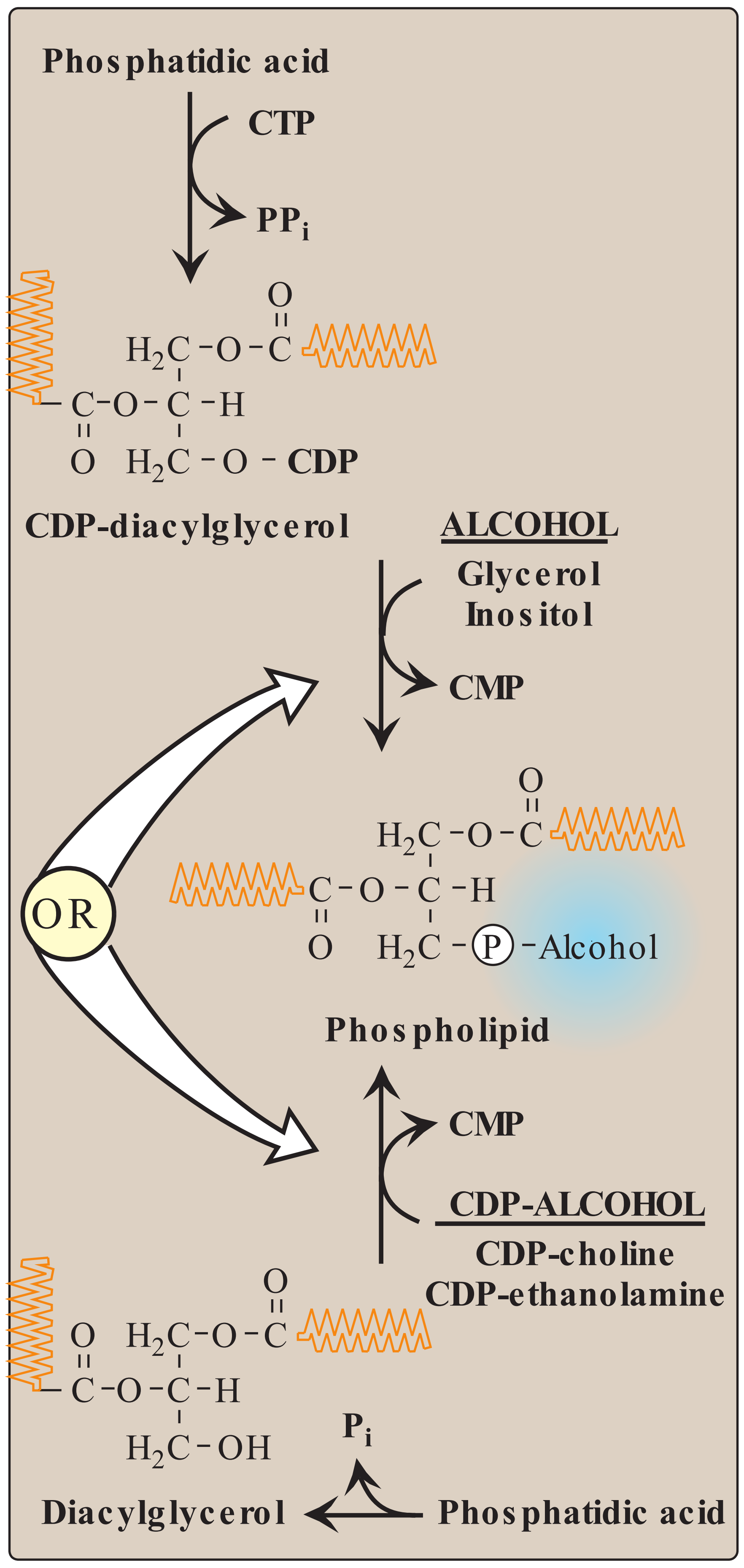


Figure 17.5
Phospholipid synthesis requires activation of either diacylglycerol or an alcohol by linkage to cytidine diphosphate (CDP). CMP = cytidine monophosphate; CTP = cytidine triphosphate; P_i = inorganic phosphate; PP_i = pyrophosphate. (〰 is a fatty acid hydrocarbon chain.)

Intake (see p. 358) of 550 mg for men and 425 mg for women. [Note: Choline is also used for the synthesis of acetylcholine, a neurotransmitter.]

b. Role of phosphatidylcholine in lung surfactant: The pathway described above is the principal pathway for the synthesis of dipalmitoylphosphatidylcholine (DPPC, or dipalmitoyl lecithin). In DPPC, positions 1 and 2 on the glycerol are occupied by palmitate. DPPC, made and secreted by type II pneumocytes, is a major lipid component of lung surfactant, which is the extracellular fluid layer lining the alveoli. Surfactant serves to decrease the surface tension of this fluid layer, reducing the pressure needed to reinflate alveoli, thereby preventing alveolar collapse (atelectasis). [Note: Surfactant is a complex mixture of lipids (90%) and proteins (10%), with DPPC being the major component for reducing surface tension.] Respiratory distress syndrome (RDS) in preterm infants is associated with insufficient surfactant production and/or secretion and is a significant cause of all neonatal deaths in Western countries.

> Lung maturity of the fetus can be gauged by determining the ratio of DPPC to sphingomyelin, usually written as the L (for lecithin)-to-S ratio, in amniotic fluid. A value of two or above is evidence of maturity, because it reflects the major shift from sphingomyelin to DPPC synthesis that occurs in the pneumocytes at about 32 weeks of gestation.

Lung maturation can be accelerated by giving the mother glucocorticoids shortly before delivery to induce expression of specific genes. Postnatal administration of natural or synthetic surfactant (by intratracheal instillation) is also used. [Note: Acute respiratory distress syndrome (ARDS), seen in all age groups, is the result of alveolar damage (due to infection, injury, or aspiration) that causes fluid to accumulate in the alveoli, impeding the exchange of oxygen and carbon dioxide.]

2. Synthesis of phosphatidylcholine from phosphatidylserine in the liver: The liver requires a mechanism for producing PC, even when free choline levels are low, because it exports significant amounts of PC in the bile and as a component of serum lipoproteins. To provide the needed PC, PS is decarboxylated to PE by PS decarboxylase, an enzyme requiring pyridoxal phosphate (PLP) as a coenzyme. PE then undergoes three methylation steps to produce PC, as illustrated in Figure 17.6. S-adenosylmethionine is the methyl group donor (see p. 264).

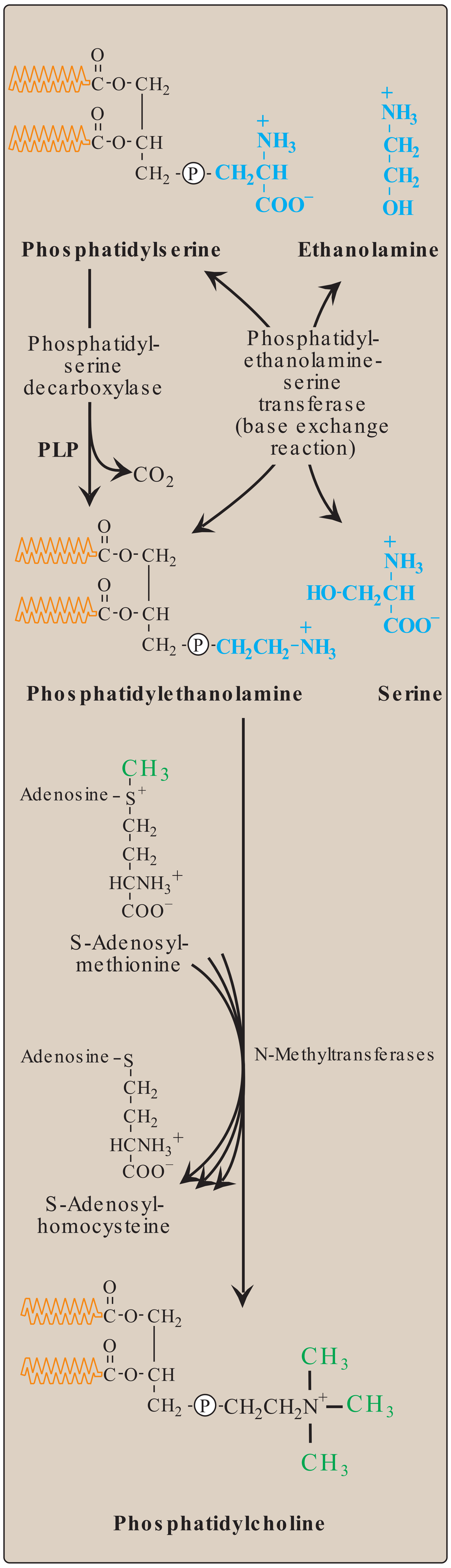


Figure 17.6
Synthesis of phosphatidylcholine from phosphatidylserine in the liver. (∿∿∿ is a fatty acid hydrocarbon chain.) Ⓟ = phosphate; PLP = pyridoxal phosphate.

C. Phosphatidylserine

PS synthesis in mammalian tissues is provided by the base exchange reaction, in which the ethanolamine of PE is exchanged for free serine (see Figure 17.6). This reaction, although reversible, is used primarily to produce the PS required for membrane synthesis.

D. Phosphatidylinositol

PI is synthesized from free inositol and CDP-diacylglycerol as shown in Figure 17.5. PI is an unusual phospholipid in that it most frequently contains stearic acid on carbon 1 and arachidonic acid on carbon 2 of the glycerol. PI, therefore, serves as a reservoir of arachidonic acid in membranes and, thus, provides the substrate for prostaglandin synthesis when required (see p. 213 for a discussion of these compounds). [Note: There is asymmetry in the phospholipid composition of the cell membrane. PS and PI, for example, are found primarily on the inner leaflet. Asymmetry is achieved by enzymes known as “flippases” and “floppases.”]

1. **Role in signal transmission across membranes:** The phosphorylation of membrane-bound PI produces polyphosphoinositides such as phosphatidylinositol 4,5-bisphosphate ([PIP$_2$] Figure 17.7). The hydrolytic cleavage of PIP$_2$ by phospholipase C occurs in response to the binding of a variety of neurotransmitters, hormones, and growth factors to G protein–coupled receptors (such as the α-1 adrenergic receptor) on the cell membrane and activation of the G$_q$ alpha subunit (Figure 17.8). The products of this cleavage, inositol 1,4,5-trisphosphate (IP$_3$) and DAG, mediate the mobilization of intracellular calcium and the activation of protein kinase C, which act synergistically to evoke specific cellular responses. Signal transmission across the membrane is thus accomplished.

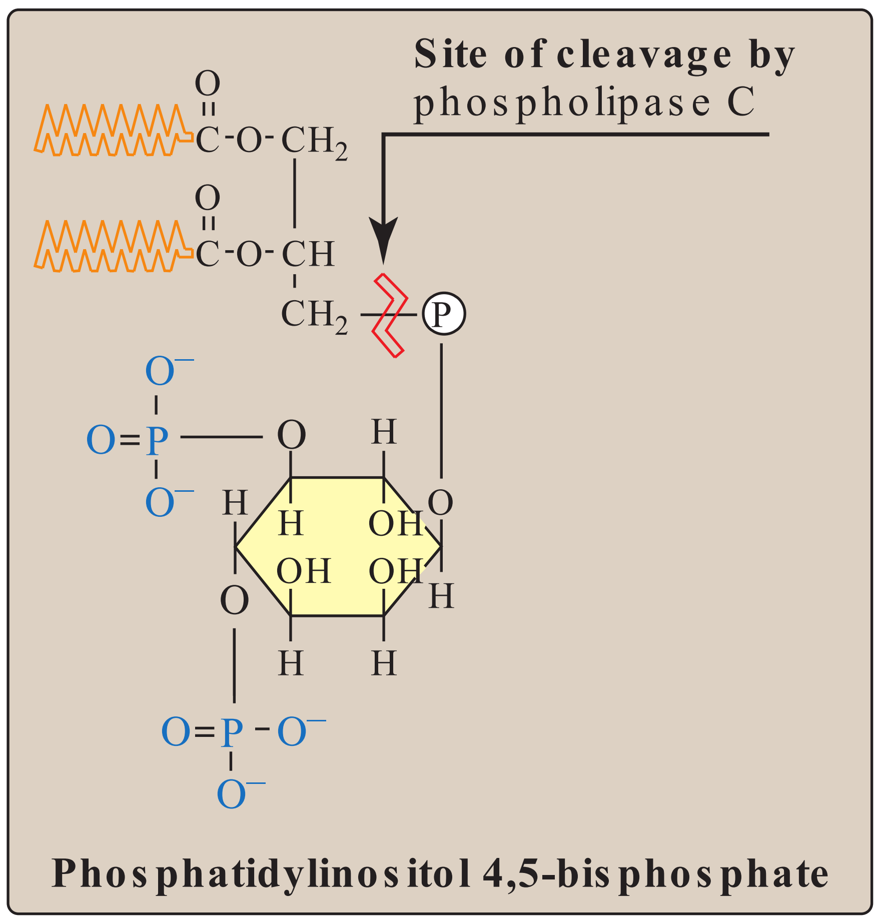
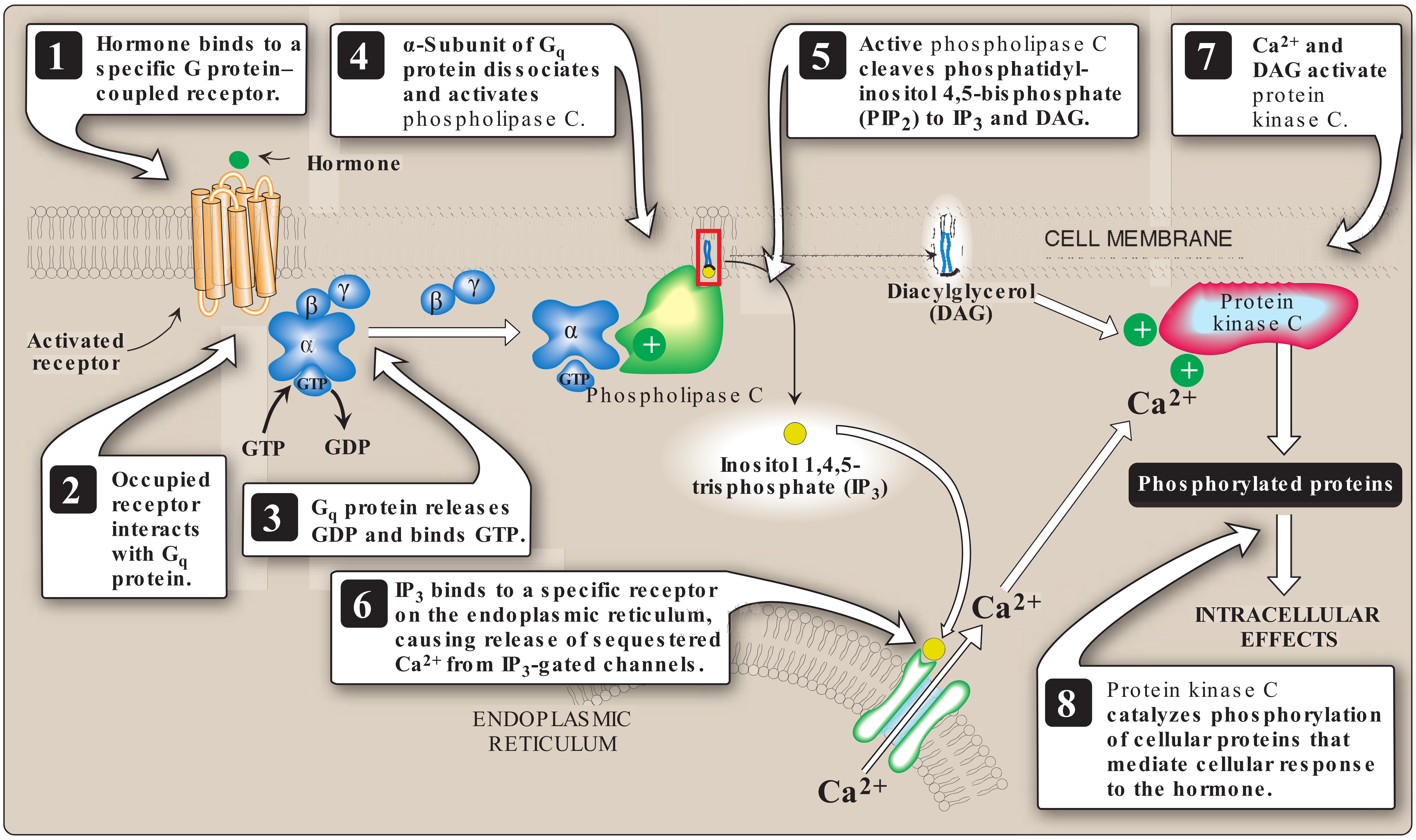

Site of cleavage by phospholipase C
O
C-O-CH2
O
C-O-CH
CH2
P
O−
O=P
O−
O
H
H
H
OH
O
OH
OH
H
O
H
H
O=P-O−
O−
Phosphatidylinositol 4,5-bisphosphate

Figure 17.7
Structure of phosphatidylinositol 4,5-bisphosphate. Cleavage by phospholipase C produces inositol 1,4,5-trisphosphate and diacylglycerol. (〰 is a fatty acid hydrocarbon chain.) Ⓟ = phosphate.


1 Hormone binds to a specific G protein–coupled receptor.

2 Occupied receptor interacts with G$_q$ protein.

3 G$_q$ protein releases GDP and binds GTP.

4 α-Subunit of G$_q$ protein dissociates and activates phospholipase C.

5 Active phospholipase C cleaves phosphatidylinositol 4,5-bisphosphate (PIP$_2$) to IP$_3$ and DAG.

6 IP$_3$ binds to a specific receptor on the endoplasmic reticulum, causing release of sequestered Ca^{2+} from IP$_3$-gated channels.

7 Ca^{2+} and DAG activate protein kinase C.

8 Protein kinase C catalyzes phosphorylation of cellular proteins that mediate cellular response to the hormone.

Hormone
Activated receptor
β
γ
α
GTP
GTP
GDP
β
γ
α
GTP
+
Phospholipase C
CELL MEMBRANE
Diacylglycerol (DAG)
+
Protein kinase C
+
Ca2+
Inositol 1,4,5-trisphosphate (IP3)
Phosphorylated proteins
INTRACELLULAR EFFECTS
Ca2+
Ca2+
ENDOPLASMIC RETICULUM

Figure 17.8
Role of inositol trisphosphate and diacylglycerol in intracellular signaling. GTP = guanosine triphosphate; GDP = guanosine diphosphate.

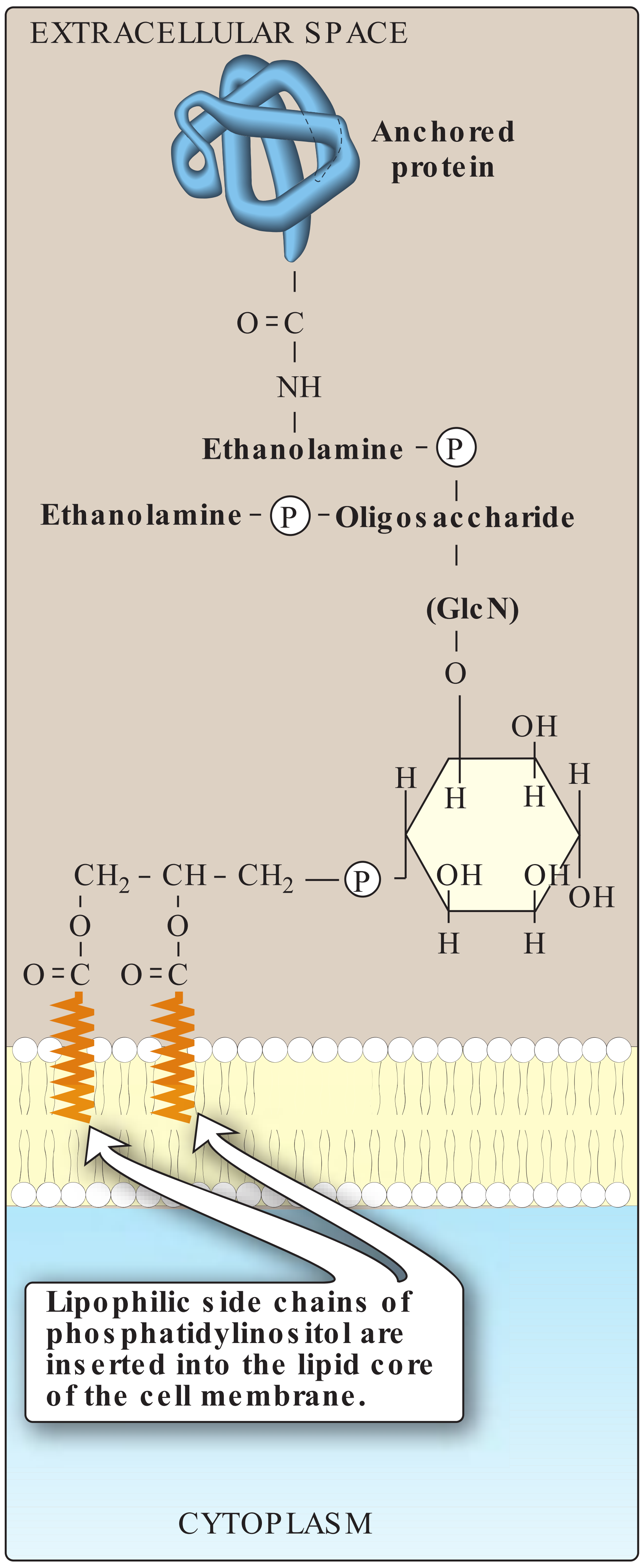

EXTRACELLULAR SPACE
Anchored protein
O=C
NH
Ethanolamine – Ⓟ
Ethanolamine – Ⓟ – Oligosaccharide
(GlcN)
O
OH
H
H
H
H
OH
OH
OH
H
H
CH2 – CH – CH2 — Ⓟ
O
O
O=C
O=C
Lipophilic side chains of phosphatidylinositol are inserted into the lipid core of the cell membrane.
CYTOPLASM

Figure 17.9
Example of a glycosyl phosphatidylinositol (GPI) membrane protein anchor. GlcN = glucosamine; Ⓟ= phosphate.

2. **Role in membrane protein anchoring:** Specific proteins can be covalently attached through a carbohydrate bridge to membrane-bound PI (Figure 17.9). Alkaline phosphatase, a digestive enzyme found on the surface of the small intestine that attacks organic phosphates, is an example of a protein attached to such a glycosyl phosphatidylinositol (GPI) anchor. [Note: GPI-linked proteins are also found in a variety of parasitic protozoans, such as, trypanosomes and leishmania.] Being attached to a membrane lipid (rather than being an integral part of the membrane) allows GPI-anchored proteins increased lateral mobility on the surface of the plasma membrane. The protein can be cleaved from its anchor by the action of phospholipase C (see Figure 17.8), releasing DAG. [Note: A deficiency in the synthesis of GPI in hematopoietic cells results in the hemolytic disease paroxysmal nocturnal hemoglobinuria.]

E. Phosphatidylglycerol and cardiolipin

Phosphatidylglycerol occurs in relatively large amounts in mitochondrial membranes and is a precursor of cardiolipin (diphosphatidylglycerol). It is synthesized by a two-step reaction from CDP-diacylglycerol and glycerol 3-phosphate. Cardiolipin (see Figure 17.2) is synthesized by the transfer of diacylglycerophosphate from CDP-diacylglycerol to a preexisting molecule of phosphatidylglycerol.

F. Sphingomyelin

Sphingomyelin, a sphingosine-based phospholipid, is a major structural lipid in the membranes of nerve tissue. The synthesis of sphingomyelin is shown in Figure 17.10. Briefly, palmitoyl CoA condenses with serine, as CoA and the carboxyl group (as CO_2) of serine are lost. [Note: This reaction, like the decarboxylation reactions involved in the synthesis of PE from PS and of regulators from amino acids (for example, the catecholamines from tyrosine, see p. 286) requires pyridoxal phosphate (a derivative of vitamin B_6) as a coenzyme (see p. 378).] The product is reduced in a nicotinamide adenine dinucleotide phosphate (NADPH)-requiring reaction to sphinganine (dihydrosphingosine), which is acylated at the amino group with one of a variety of long-chain fatty acids and then desaturated to produce a ceramide, the immediate precursor of sphingomyelin (and other sphingolipids, as described on p. 208).

> Ceramides play a key role in maintaining the skin's water-permeability barrier. Decreased ceramide levels are associated with a number of skin diseases.

Phosphorylcholine from PC is transferred to the ceramide, producing sphingomyelin and DAG. [Note: Sphingomyelin of the myelin sheath contains predominantly longer-chain fatty acids such as lignoceric acid and nervonic acid, whereas gray matter of the brain has sphingomyelin that contains primarily stearic acid.]

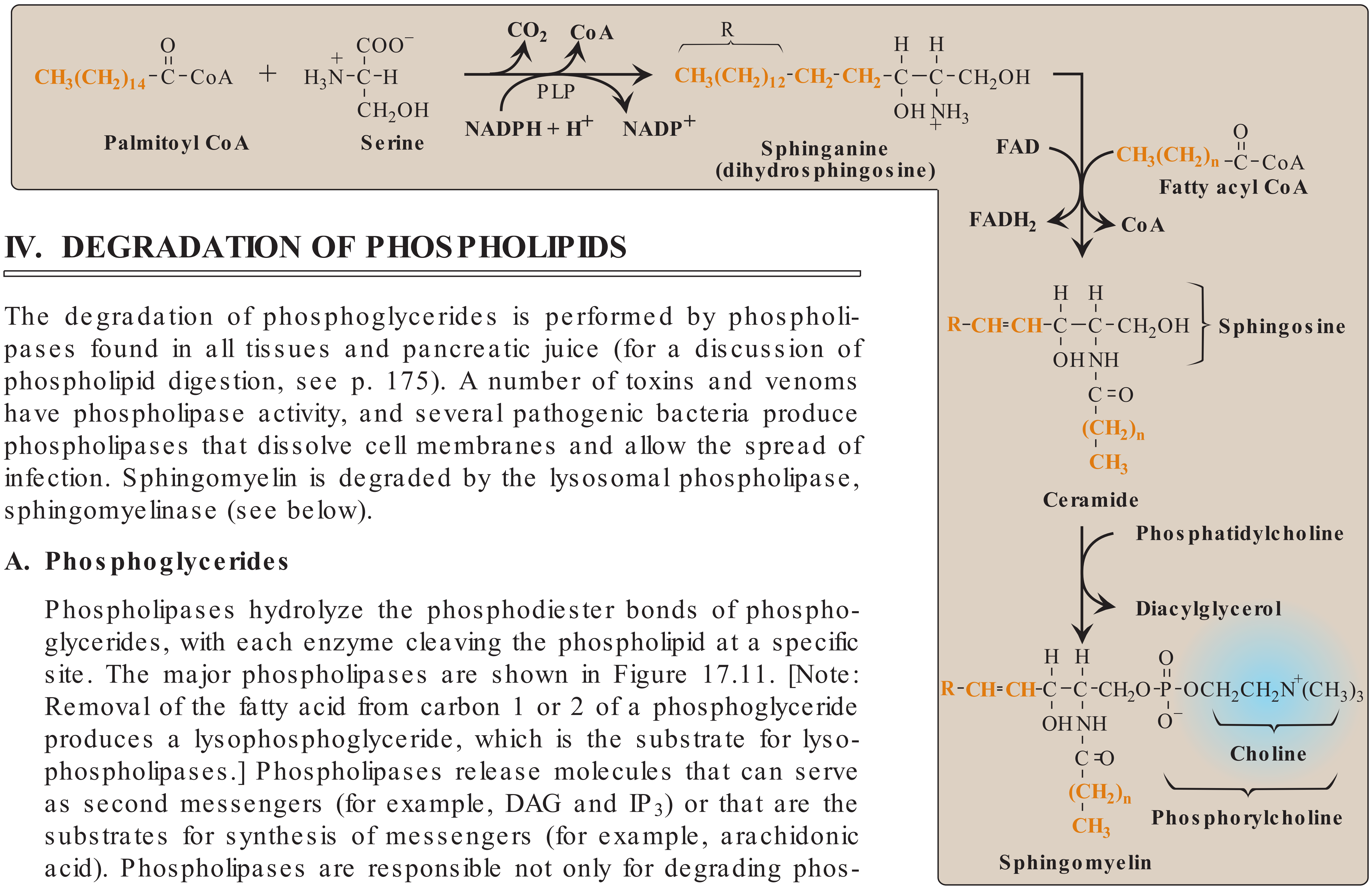

$CH_3(CH_2)_{14}$-C(=O)-CoA
Palmitoyl CoA
+
$H_3\overset{+}{N}$-CH(COO⁻)-CH_2OH
Serine
CO_2
CoA
PLP
NADPH + H^+
$NADP^+$
R
$CH_3(CH_2)_{12}$-CH_2-CH_2-CH(OH)-CH($\overset{+}{N}H_3$)-CH_2OH
Sphinganine (dihydrosphingosine)
FAD
$FADH_2$
$CH_3(CH_2)_n$-C(=O)-CoA
Fatty acyl CoA
CoA
R-CH=CH-CH(OH)-CH(NH)-CH_2OH
Sphingosine
C=O
$(CH_2)_n$
CH_3
Ceramide
Phosphatidylcholine
Diacylglycerol
R-CH=CH-CH(OH)-CH(NH)-CH_2O-P(=O)(O⁻)-$OCH_2CH_2\overset{+}{N}(CH_3)_3$
Choline
Phosphorylcholine
Sphingomyelin

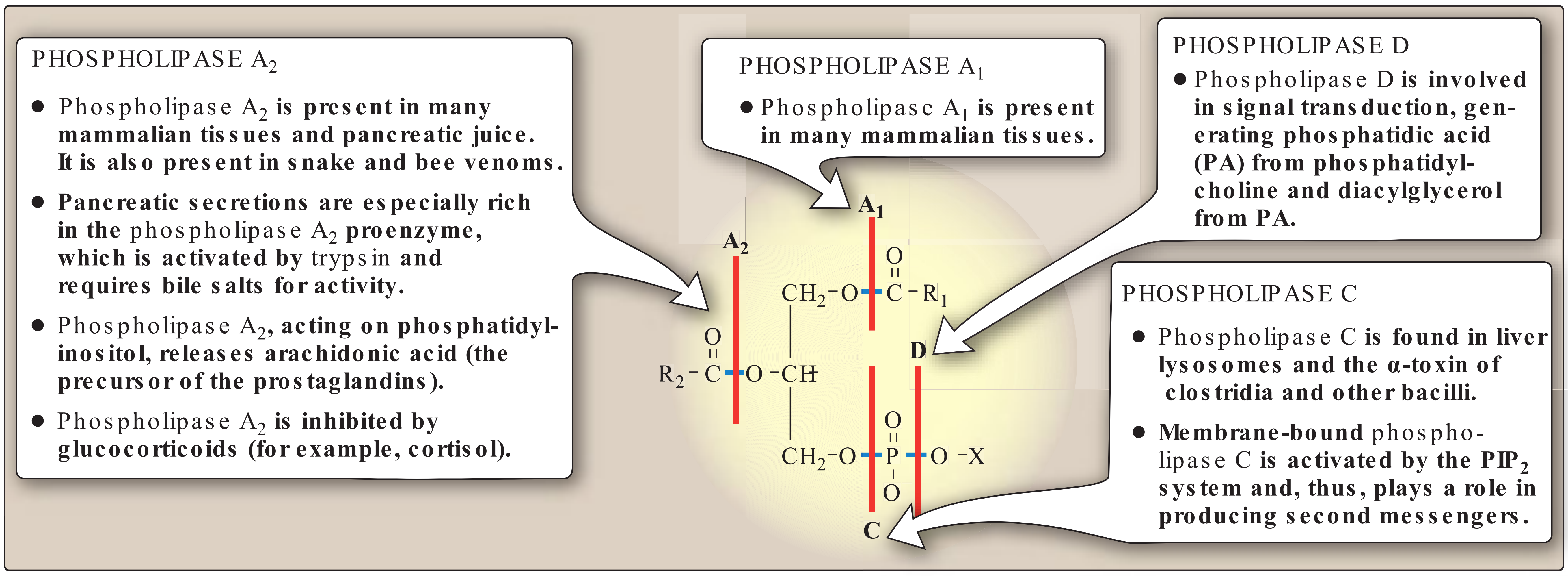
Figure 17.10
Synthesis of sphingomyelin. PLP = pyridoxal phosphate; NADP(H) = nicotinamide adenine dinucleotide phosphate; FAD(H_2) = flavin adenine dinucleotide; CoA = coenzyme A.

IV. DEGRADATION OF PHOSPHOLIPIDS

The degradation of phosphoglycerides is performed by phospholipases found in all tissues and pancreatic juice (for a discussion of phospholipid digestion, see p. 175). A number of toxins and venoms have phospholipase activity, and several pathogenic bacteria produce phospholipases that dissolve cell membranes and allow the spread of infection. Sphingomyelin is degraded by the lysosomal phospholipase, sphingomyelinase (see below).

A. Phosphoglycerides

Phospholipases hydrolyze the phosphodiester bonds of phosphoglycerides, with each enzyme cleaving the phospholipid at a specific site. The major phospholipases are shown in Figure 17.11. [Note: Removal of the fatty acid from carbon 1 or 2 of a phosphoglyceride produces a lysophosphoglyceride, which is the substrate for lysophospholipases.] Phospholipases release molecules that can serve as second messengers (for example, DAG and IP_3) or that are the substrates for synthesis of messengers (for example, arachidonic acid). Phospholipases are responsible not only for degrading phospholipids, but also for "remodeling" them. For example, phospholipases A_1 and A_2 remove specific fatty acids from membrane-bound phospholipids, which can be replaced with different fatty acids using fatty acyl CoA transferase. This mechanism is used as one way to create the unique lung surfactant DPCC (see p. 204) and to insure that carbon 2 of PI (and sometimes of PC) is bound to arachidonic


A_2
A_1
D
C
CH_2-O-C(=O)-R_1
R_2-C(=O)-O-CH
CH_2-O-P(=O)(O⁻)-O-X

PHOSPHOLIPASE A_2

- Phospholipase A_2 **is present in many mammalian tissues and pancreatic juice. It is also present in snake and bee venoms.**
- **Pancreatic secretions are especially rich in the** phospholipase A_2 **proenzyme, which is activated by** trypsin **and requires bile salts for activity.**
- Phospholipase A_2, **acting on phosphatidylinositol, releases arachidonic acid (the precursor of the prostaglandins).**
- Phospholipase A_2 **is inhibited by glucocorticoids (for example, cortisol).**

PHOSPHOLIPASE A_1

- Phospholipase A_1 **is present in many mammalian tissues.**

PHOSPHOLIPASE D

- Phospholipase D **is involved in signal transduction, generating phosphatidic acid (PA) from phosphatidylcholine and diacylglycerol from PA.**

PHOSPHOLIPASE C

- Phospholipase C **is found in liver lysosomes and the α-toxin of clostridia and other bacilli.**
- **Membrane-bound** phospholipase C **is activated by the PIP_2 system and, thus, plays a role in producing second messengers.**

Figure 17.11
Degradation of glycerophospholipids by phospholipases. PIP_2 = phosphatidylinositol 4,5-bisphosphate; R_1 and R_2 = fatty acids; X = an alcohol.

acid. [Note: Barth syndrome, a rare X-linked disorder characterized by cardiomyopathy, muscle weakness, and neutropenia, is the result of defects in cardiolipin remodeling.]

B. Sphingomyelin

Sphingomyelin is degraded by sphingomyelinase, a lysosomal enzyme that hydrolytically removes phosphorylcholine, leaving a ceramide. The ceramide is, in turn, cleaved by ceramidase into sphingosine and a free fatty acid (Figure 17.12). [Note: The ceramide and sphingosine released regulate signal transduction pathways, in part by influencing the activity of protein kinase C and, thus, the phosphorylation of its protein substrates. They also promote apoptosis.] Niemann-Pick disease (Types A and B) is an autosomal-recessive disease caused by the inability to degrade sphingomyelin due to a deficiency of sphingomyelinase, a type of phospholipase C. In the severe infantile form (Type A, which shows less than 1% of normal enzymic activity), the liver and spleen are the primary sites of lipid deposits and are, therefore, greatly enlarged. The lipid consists primarily of the sphingomyelin that cannot be degraded (Figure 17.13). Infants with this lysosomal storage disease experience rapid and progressive neurodegeneration as a result of deposition of sphingomyelin in the CNS, and they die in early childhood. A less severe variant (Type B, which shows 5% or more of normal activity) with a later age of onset and a longer survival time causes little to no damage to neural tissue, but lungs, spleen, liver, and bone marrow are affected, resulting in a chronic form of the disease. Although Niemann-Pick disease occurs in all ethnic groups, Type A occurs with greater frequency in the Ashkenazi Jewish population.

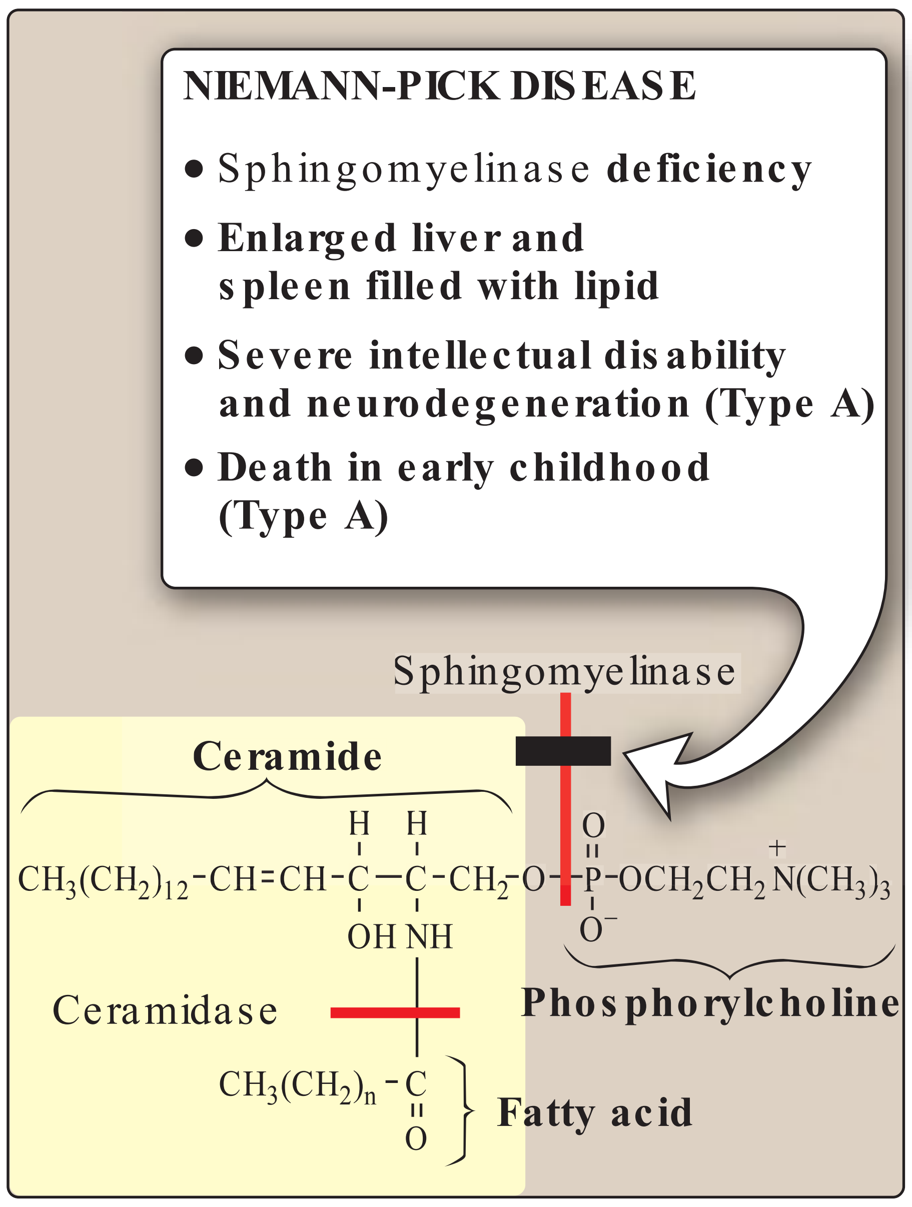

NIEMANN-PICK DISEASE
• Sphingomyelinase deficiency
• Enlarged liver and spleen filled with lipid
• Severe intellectual disability and neurodegeneration (Type A)
• Death in early childhood (Type A)
Sphingomyelinase
Ceramide
CH3(CH2)12-CH=CH-C-C-CH2-O-P-OCH2CH2N(CH3)3
H H
OH NH
O
O−
+
Ceramidase
Phosphorylcholine
CH3(CH2)n-C
O
Fatty acid

Figure 17.12
Degradation of sphingomyelin. [Note: Type B is the nonneuropathic form. It has a later age of onset and a longer survival time than Type A.]

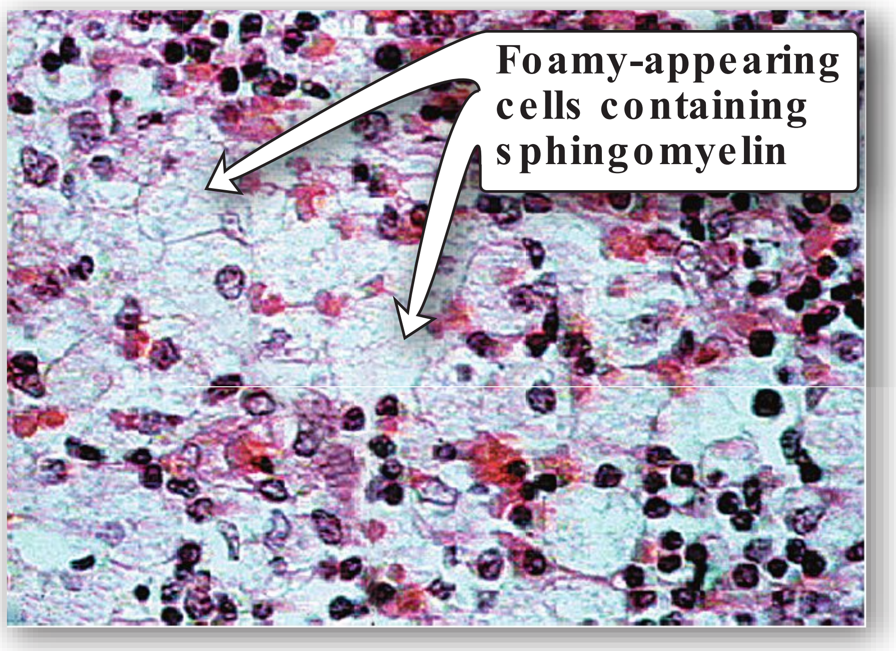

Foamy-appearing cells containing sphingomyelin

Figure 17.13
Accumulation of lipids in spleen cells from a patient with Niemann-Pick disease.

V. OVERVIEW OF GLYCOLIPIDS

Glycolipids are molecules that contain both carbohydrate and lipid components. Like the phospholipid sphingomyelin, glycolipids are derivatives of ceramides in which a long-chain fatty acid is attached to the amino alcohol sphingosine. They are, therefore, more precisely called glycosphingolipids. [Note: Ceramides, then, are the precursors of both phosphorylated and glycosylated sphingolipids.] Like the phospholipids, glycosphingolipids are essential components of all membranes in the body, but they are found in greatest amounts in nerve tissue. They are located in the outer leaflet of the plasma membrane, where they interact with the extracellular environment. As such, they play a role in the regulation of cellular interactions (for example, adhesion and recognition), growth, and development.

Membrane glycosphingolipids associate with cholesterol and GPI-anchored proteins to form lipid rafts, laterally mobile microdomains of the plasma membrane that function to organize and regulate signaling and trafficking functions of membranes.

Glycosphingolipids are antigenic and are the source of blood group antigens, various embryonic antigens specific for particular stages of fetal development, and some tumor antigens. [Note: The carbohydrate portion of a glycolipid is the antigenic determinant.] They also are used as cell surface receptors for cholera and tetanus toxins as well as for certain viruses and microbes. Genetic disorders associated with an inability to properly degrade the glycosphingolipids result in lysosomal accumulation of these compounds. [Note: Changes in the carbohydrate portion of glycosphingolipids (and glycoproteins) are characteristic of transformed cells (cells with dysregulated growth).]

VI. STRUCTURE OF GLYCOSPHINGOLIPIDS

The glycosphingolipids differ from sphingomyelin in that they do not contain phosphate, and the polar head function is provided by a monosaccharide or oligosaccharide attached directly to the ceramide by an O-glycosidic bond (Figure 17.14). The number and type of carbohydrate moieties present determine the type of glycosphingolipid.

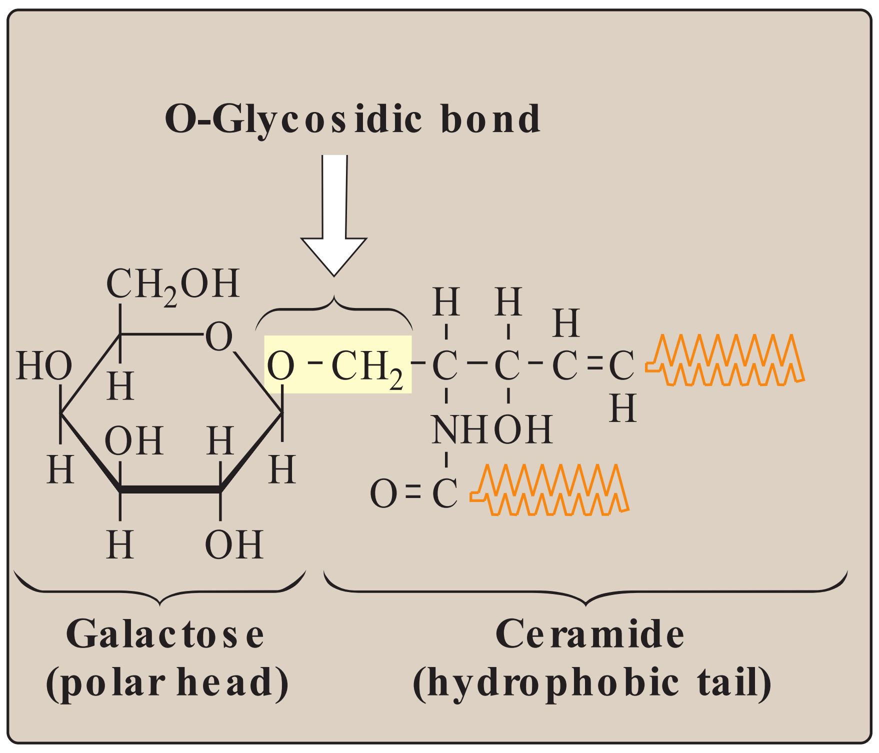


Figure 17.14
Structure of a neutral glycosphingolipid, galactocerebroside. (〰 is a hydrophobic hydrocarbon chain.)

A. Neutral glycosphingolipids

The simplest neutral (uncharged) glycosphingolipids are the cerebrosides. These are ceramide monosaccharides that contain either a molecule of galactose (forming ceramide-galactose or galactocerebroside, the most common cerebroside found in myelin, as shown in Figure 17.14) or glucose (forming ceramide-glucose or glucocerebroside, which serves primarily as an intermediate in the synthesis and degradation of the more complex glycosphingolipids). [Note: Members of a group of galacto- or glucocerebrosides may also differ from each other in the type of fatty acid attached to the sphingosine.] As their name implies, cerebrosides are found predominantly in the brain and peripheral nervous tissue, with high concentrations in the myelin sheath. Ceramide oligosaccharides (or globosides) are produced by attaching additional monosaccharides to a glucocerebroside, for example, ceramide-glucose-galactose (also known as lactosylceramide). The additional monosaccharides can include substituted sugars such as N-acetylgalactosamine.

B. Acidic glycosphingolipids

Acidic glycosphingolipids are negatively charged at physiologic pH. The negative charge is provided by N-acetylneuraminic acid ([NANA], a sialic acid, as shown in Figure 17.15) in gangliosides, or by sulfate groups in sulfatides.

1. **Gangliosides:** These are the most complex glycosphingolipids and are found primarily in the ganglion cells of the CNS, particularly at the nerve endings. They are derivatives of ceramide oligosaccharides and contain one or more molecules of NANA. The notation for these compounds is G (for ganglioside) plus a subscript M, D, T, or Q to indicate whether there is one (mono), two (di), three (tri), or four (quatro) molecules of NANA in the ganglioside, respectively. Additional numbers and letters in the subscript designate the monomeric sequence of the carbohydrate attached to the ceramide. (See Figure 17.15 for the structure of G_{M2}.) Gangliosides are of medical interest because several lipid storage disorders involve the accumulation of NANA-containing glycosphingolipids in cells (see Figure 17.20, p. 212).

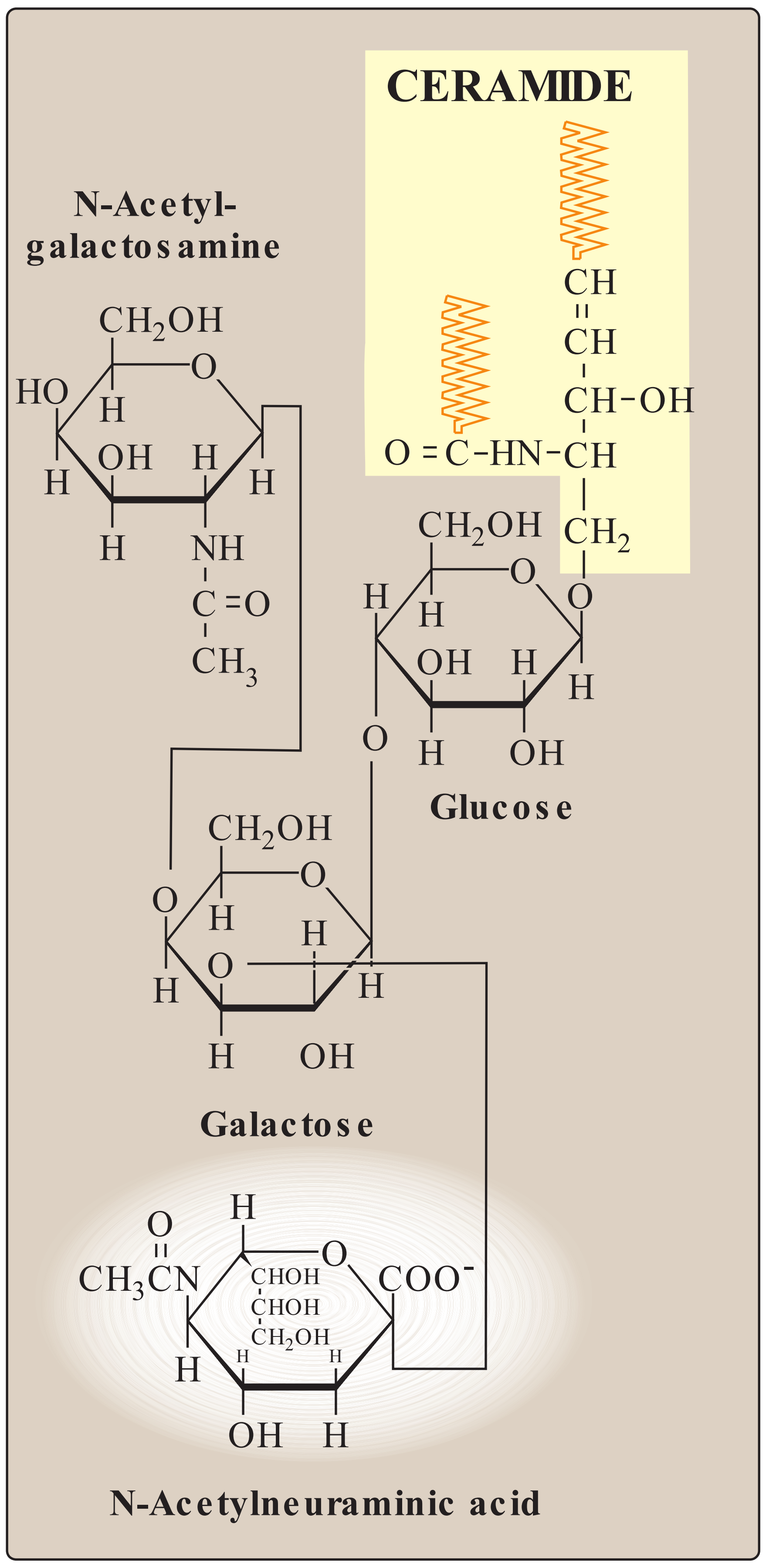


Figure 17.15
Structure of the ganglioside G_{M2}. (〰 is a hydrophobic hydrocarbon chain.)

2. **Sulfatides:** These sulfoglycosphingolipids are sulfated galactocerebrosides that are negatively charged at physiologic pH. Sulfatides are found predominantly in the brain and kidneys.

VII. SYNTHESIS AND DEGRADATION OF GLYCOSPHINGOLIPIDS

Synthesis of glycosphingolipids occurs primarily in the Golgi by sequential addition of glycosyl monomers transferred from UDP-sugar donors to the acceptor molecule. The mechanism is similar to that used in glycoprotein synthesis (see p. 166).

A. Enzymes involved in synthesis

The enzymes involved in the synthesis of glycosphingolipids are glycosyltransferases that are specific for the type and location of the glycosidic bond formed. [Note: These enzymes can recognize both glycosphingolipids and glycoproteins as substrates.]

B. Addition of sulfate groups

A sulfate group from the sulfate carrier 3'-phosphoadenosine-5'-phosphosulfate ([PAPS], Figure 17.16) is added by a sulfotransferase to the 3'-hydroxyl group of the galactose in a galactocerebroside, forming the sulfatide galactocerebroside 3-sulfate (Figure 17.17). [Note: PAPS is also the sulfur donor in glycosaminoglycan synthesis (see p. 162) and steroid hormone catabolism (see p. 240).] An overview of the synthesis of sphingolipids is shown in Figure 17.18.

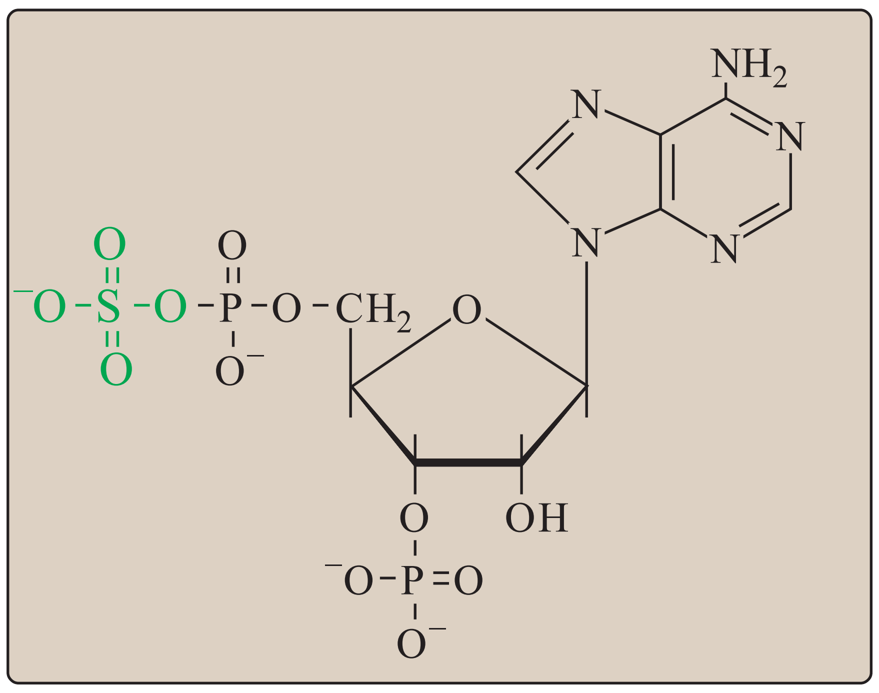


Figure 17.16
Structure of 3'-phosphoadenosine-5'-phosphosulfate.

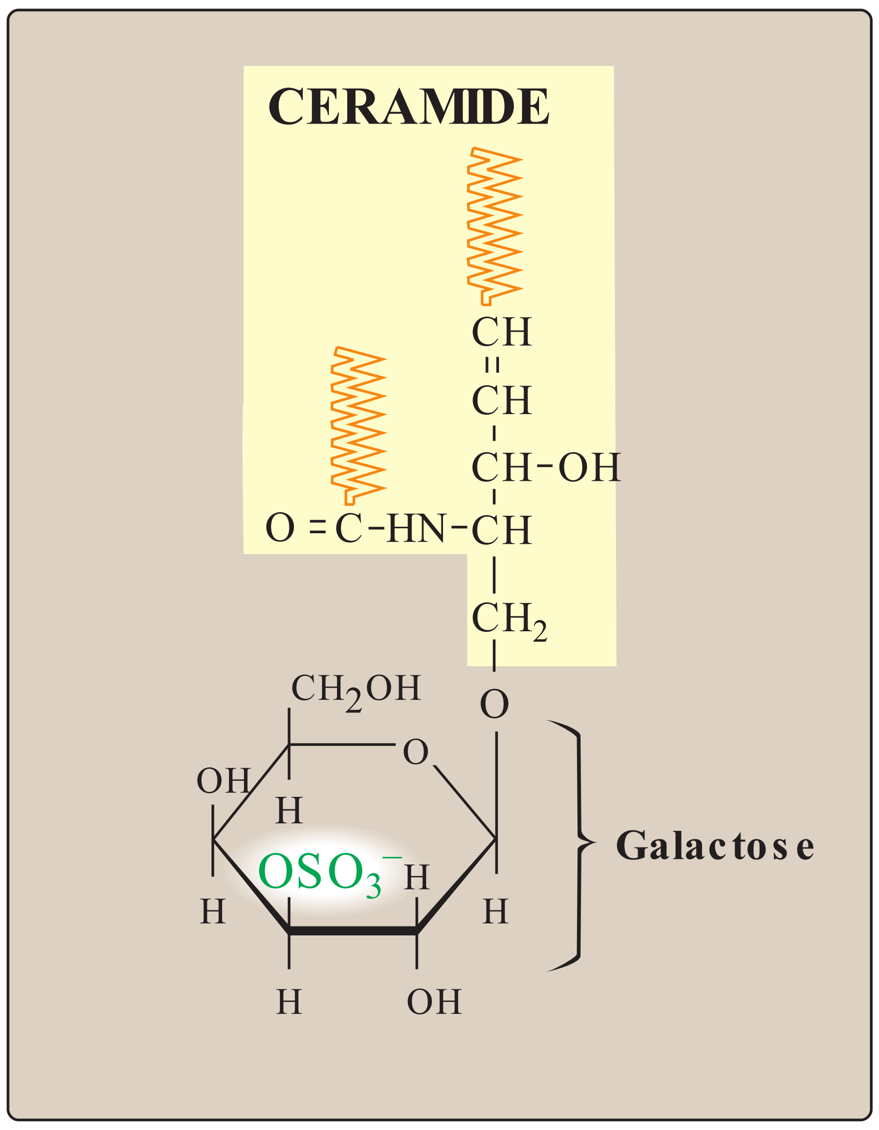


Figure 17.17
Structure of galactocerebroside 3-sulfate. (〰 is a hydrophobic hydrocarbon chain.)

C. Degradation of glycosphingolipids

Glycosphingolipids are internalized by endocytosis as described for the glycosaminoglycans. All of the enzymes required for the degradative process are present in lysosomes, which fuse with the endocytotic vesicles. The lysosomal enzymes hydrolytically and irreversibly cleave specific bonds in the glycosphingolipid. As seen with the glycosaminoglycans (see p. 163) and glycoproteins (see p. 170), degradation is a sequential process following the rule "last on, first off," in which the last group added during synthesis is the first group removed in degradation. [Note: Defects in the degradation of the polysaccharide chains in these three glycoconjugates, therefore, result in lysosomal storage diseases.]

D. Sphingolipidoses

In a normal individual, synthesis and degradation of glycosphingolipids are balanced, so that the amount of these compounds present in membranes is constant. If a specific lysosomal acid hydrolase required for degradation is partially or totally missing, a sphingolipid accumulates. Lysosomal lipid storage diseases caused by these deficiencies are called sphingolipidoses. The result of a specific acid hydrolase deficiency may be seen dramatically in nerve tissue, where neurologic deterioration can lead to early death. Figure 17.20 provides an outline of the pathway of sphingolipid degradation and descriptions of some sphingolipidoses. [Note: Some sphingolipidoses can also result from defects in lysosomal activator proteins (for

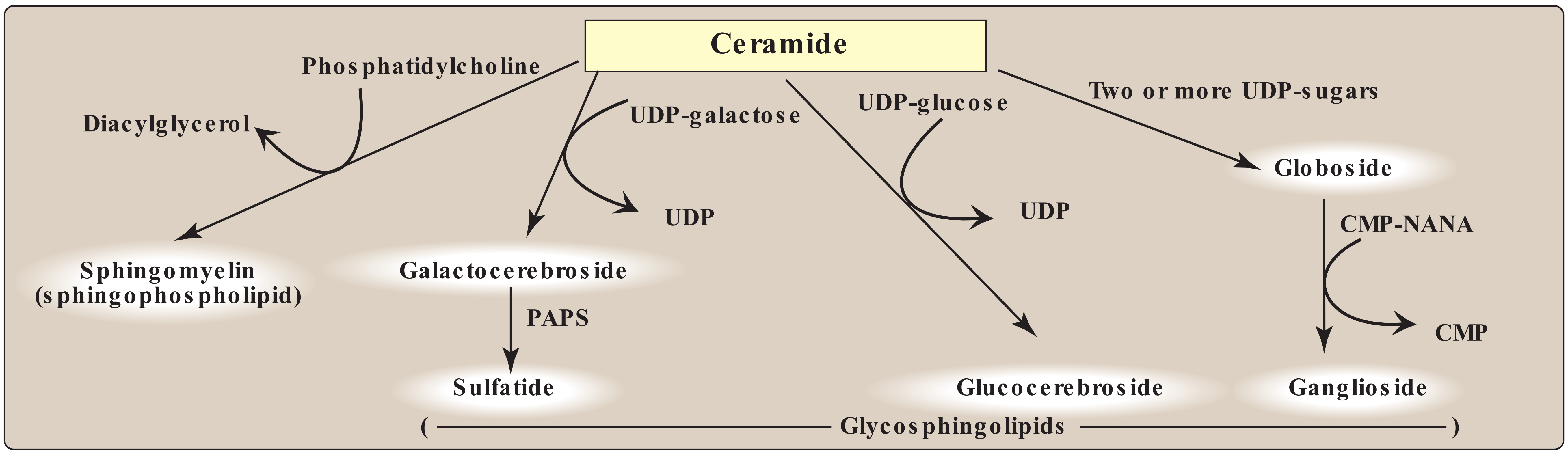


Figure 17.18
Overview of sphingolipid synthesis. UDP = uridine diphosphate; CMP = cytidine monophosphate; NANA = N-acetylneuraminic acid; PAPS = 3'-phosphoadenosine-5'-phosphosulfate.

example, the saposins) that facilitate access of the hydrolases to short carbohydrate chains as degradation proceeds.]

1. **Common properties:** A specific lysosomal hydrolytic enzyme is deficient in the classic form of each disorder. Therefore, usually only a single sphingolipid (the substrate for the deficient enzyme) accumulates in the involved organs in each disease. [Note: The rate of biosynthesis of the accumulating lipid is normal.] The disorders are progressive and, although many are fatal in childhood, extensive phenotypic variability is seen leading to the designation of different clinical types, such as Types A and B in Niemann-Pick disease. Genetic variability is also seen because a given disorder can be caused by any one of a variety of mutations within a single gene. The sphingolipidoses are autosomal-recessive diseases, except for Fabry disease, which is X linked. The incidence of the sphingolipidoses is low in most populations, except for Gaucher and Tay-Sachs diseases, which, like Niemann-Pick disease, show a high frequency in the Ashkenazi Jewish population. [Note: Tay-Sachs also has a high frequency in Irish American, French Canadian, and Louisiana Cajun populations.]

2. **Diagnosis and treatment:** A specific sphingolipidosis can be diagnosed by measuring enzyme activity in cultured fibroblasts or peripheral leukocytes or by analysis of DNA (see p. 473). Histologic examination of the affected tissue is also useful. [Note: Shell-like inclusion bodies are seen in Tay-Sachs, and a wrinkled tissue paper appearance of the cytosol is seen in Gaucher disease (Figure 17.19).] Prenatal diagnosis, using cultured amniocytes or chorionic villi, is available. Gaucher disease, in which macrophages become engorged with glucocerebroside, and Fabry disease, in which globosides accumulate in the vascular endothelial lysosomes of the brain, heart, kidneys, and skin, are treated by recombinant human enzyme replacement therapy, but the monetary cost is extremely high. Gaucher has also been treated by bone marrow transplantation (because macrophages are derived from hematopoietic stem cells) and by substrate reduction therapy through pharmacologic reduction of glucosylceramide, the substrate for the deficient enzyme.

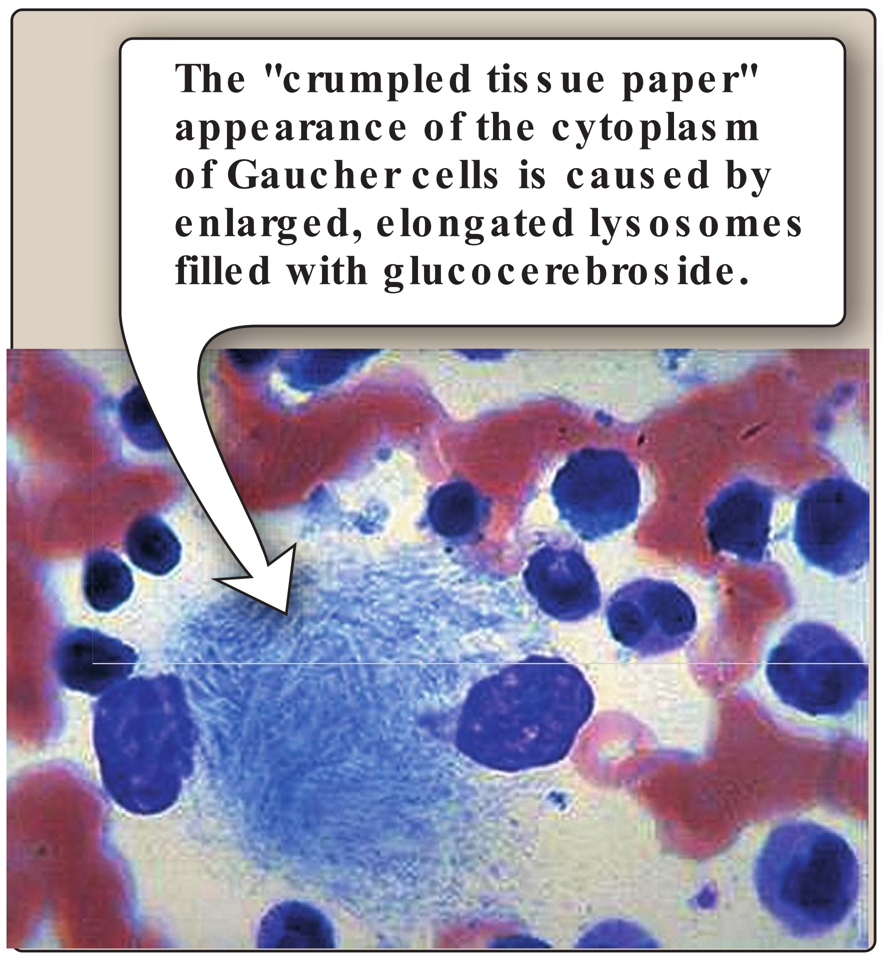
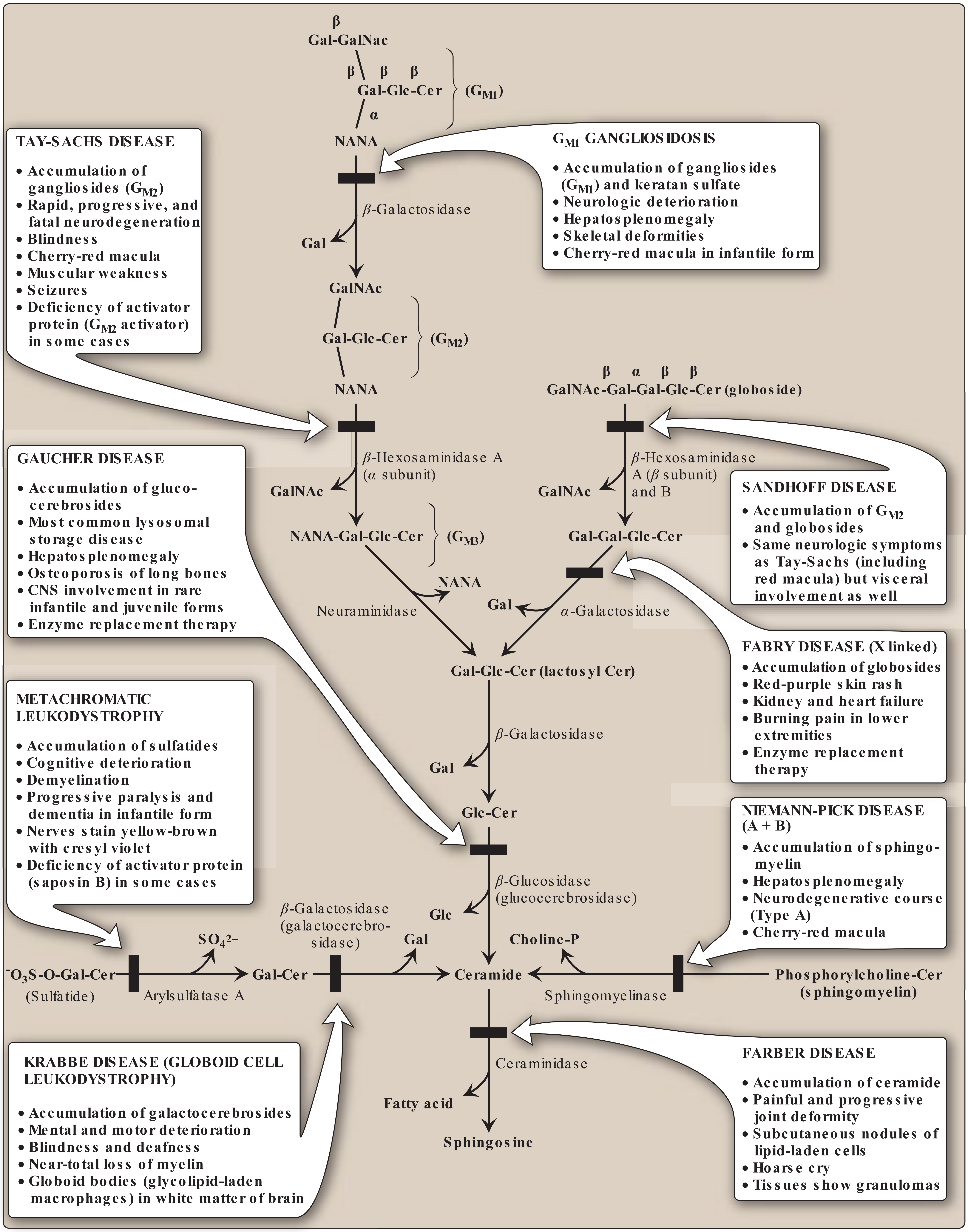

The "crumpled tissue paper" appearance of the cytoplasm of Gaucher cells is caused by enlarged, elongated lysosomes filled with glucocerebroside.

Figure 17.19
Aspirated bone marrow cells from a patient with Gaucher disease.


β
Gal-GalNac
β β β
Gal-Glc-Cer
α
NANA
(G_{M1})
β-Galactosidase
Gal
GalNAc
Gal-Glc-Cer
NANA
(G_{M2})
β-Hexosaminidase A (α subunit)
GalNAc
NANA-Gal-Glc-Cer
(G_{M3})
β α β β
GalNAc-Gal-Gal-Glc-Cer (globoside)
β-Hexosaminidase A (β subunit) and B
GalNAc
Gal-Gal-Glc-Cer
NANA
Neuraminidase
Gal
α-Galactosidase
Gal-Glc-Cer (lactosyl Cer)
β-Galactosidase
Gal
Glc-Cer
β-Glucosidase (glucocerebrosidase)
Glc
$^{-}O_3S$-O-Gal-Cer
(Sulfatide)
SO_4^{2-}
Arylsulfatase A
Gal-Cer
β-Galactosidase (galactocerebrosidase)
Gal
Ceramide
Choline-P
Sphingomyelinase
Phosphorylcholine-Cer (sphingomyelin)
Ceraminidase
Fatty acid
Sphingosine

TAY-SACHS DISEASE

- Accumulation of gangliosides (G_{M2})
- Rapid, progressive, and fatal neurodegeneration
- Blindness
- Cherry-red macula
- Muscular weakness
- Seizures
- Deficiency of activator protein (G_{M2} activator) in some cases

G_{M1} GANGLIOSIDOSIS

- Accumulation of gangliosides (G_{M1}) and keratan sulfate
- Neurologic deterioration
- Hepatosplenomegaly
- Skeletal deformities
- Cherry-red macula in infantile form

GAUCHER DISEASE

- Accumulation of glucocerebrosides
- Most common lysosomal storage disease
- Hepatosplenomegaly
- Osteoporosis of long bones
- CNS involvement in rare infantile and juvenile forms
- Enzyme replacement therapy

SANDHOFF DISEASE

- Accumulation of G_{M2} and globosides
- Same neurologic symptoms as Tay-Sachs (including red macula) but visceral involvement as well

FABRY DISEASE (X linked)

- Accumulation of globosides
- Red-purple skin rash
- Kidney and heart failure
- Burning pain in lower extremities
- Enzyme replacement therapy

METACHROMATIC LEUKODYSTROPHY

- Accumulation of sulfatides
- Cognitive deterioration
- Demyelination
- Progressive paralysis and dementia in infantile form
- Nerves stain yellow-brown with cresyl violet
- Deficiency of activator protein (saposin B) in some cases

NIEMANN-PICK DISEASE (A + B)

- Accumulation of sphingomyelin
- Hepatosplenomegaly
- Neurodegenerative course (Type A)
- Cherry-red macula

KRABBE DISEASE (GLOBOID CELL LEUKODYSTROPHY)

- Accumulation of galactocerebrosides
- Mental and motor deterioration
- Blindness and deafness
- Near-total loss of myelin
- Globoid bodies (glycolipid-laden macrophages) in white matter of brain

FARBER DISEASE

- Accumulation of ceramide
- Painful and progressive joint deformity
- Subcutaneous nodules of lipid-laden cells
- Hoarse cry
- Tissues show granulomas

Figure 17.20
Degradation of sphingolipids showing the lysosomal enzymes affected in related genetic diseases, the sphingolipidoses. All of the diseases are autosomal recessive except Fabry disease, which is X linked, and all can be fatal in early life. Cer = ceramide; Gal = galactose; Glc = glucose; GalNAc = N-acetylgalactosamine; NANA = N-acetylneuraminic acid; CNS = central nervous system. SO_4^{2-} = sulfate.

VIII. EICOSANOIDS: PROSTAGLANDINS AND RELATED COMPOUNDS

Prostaglandins, and the related compounds thromboxanes and leukotrienes, are collectively known as eicosanoids to reflect their origin from polyunsaturated fatty acids with 20 carbons (eicosa = 20). They are extremely potent compounds that elicit a wide range of responses, both physiologic (inflammatory response) and pathologic (hypersensitivity). They ensure gastric integrity and renal function, regulate smooth muscle contraction (intestine and uterus are key sites) and blood vessel diameter, and maintain platelet homeostasis. Although they have been compared to hormones in terms of their actions, eicosanoids differ from endocrine hormones in that they are produced in very small amounts in almost all tissues rather than in specialized glands. They also act locally rather than after transport in the blood to distant sites, as occurs with endocrine hormones such as insulin. Eicosanoids are not stored, and they have an extremely short half-life, being rapidly metabolized to inactive products. Their biologic actions are mediated by plasma membrane G protein–coupled receptors (see p. 94), which are different in different organ systems, and typically result in changes in cyclic adenosine monophosphate production. Examples of prostaglandins and related structures are shown in Figure 17.21.

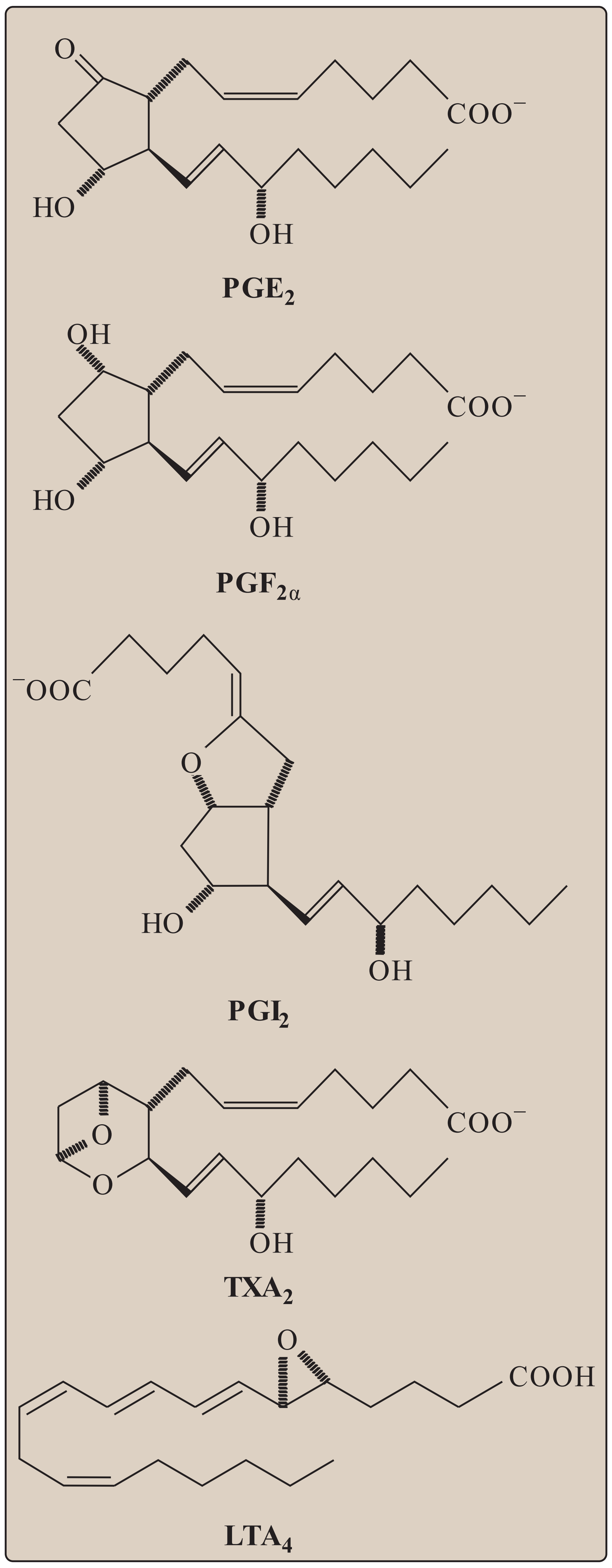


Figure 17.21
Examples of prostaglandin structures. [Note: Prostaglandins are named as follows: PG plus a third letter (for example, A, D, E, or F), which designates the type and arrangement of functional groups in the molecule. The subscript number indicates the number of double bonds in the molecule. PGI_2 is known as prostacyclin. Thromboxanes are designated by TX and leukotrienes by LT.]

A. Synthesis of prostaglandins and thromboxanes

Arachidonic acid, an ω-6 fatty acid containing 20 carbons and four double bonds (an eicosatetraenoic fatty acid), is the immediate precursor of the predominant type of prostaglandins in humans (series 2 or those with two double bonds, as shown in Figure 17.22). It is derived by the elongation and desaturation of the essential fatty acid linoleic acid, also an ω-6 fatty acid. Arachidonic acid is incorporated into membrane phospholipids (typically PI) at carbon 2, from which it is released by phospholipase A_2 in response to a variety of signals (Figure 17.23). [Note: Series 1 prostaglandins contain one double bond and are derived from an ω-6 eicosatrienoic fatty acid, dihomo-γ-linolenic acid, whereas series 3 contain three double bonds and are derived from eicosapentaenoic acid (EPA), an ω-3 fatty acid. See p. 363.]

1. **Synthesis of PGH_2:** The first step in prostaglandin synthesis is the oxidative cyclization of free arachidonic acid to yield PGH_2 by prostaglandin endoperoxide synthase (PGH synthase). This enzyme is an ER membrane-bound protein that has two catalytic activities: fatty acid cyclooxygenase (COX), which requires two molecules of O_2, and peroxidase, which is dependent on reduced glutathione (see p. 148). PGH_2 is converted to a variety of prostaglandins and thromboxanes, as shown in Figure 17.23, by cell-specific synthases.

   a. **Isozymes of PGH synthase:** Two isozymes of PGH synthase, usually denoted as COX-1 and COX-2, are known. COX-1 is made constitutively in most tissues and is required for maintenance of healthy gastric tissue, renal homeostasis, and platelet aggregation. COX-2 is inducible in a limited number of tissues in response to products of activated immune and inflammatory cells. [Note: The increase in prostaglandin synthesis subsequent to the induction of COX-2 mediates the pain, heat, redness, and swelling of inflammation and the fever of infection.]

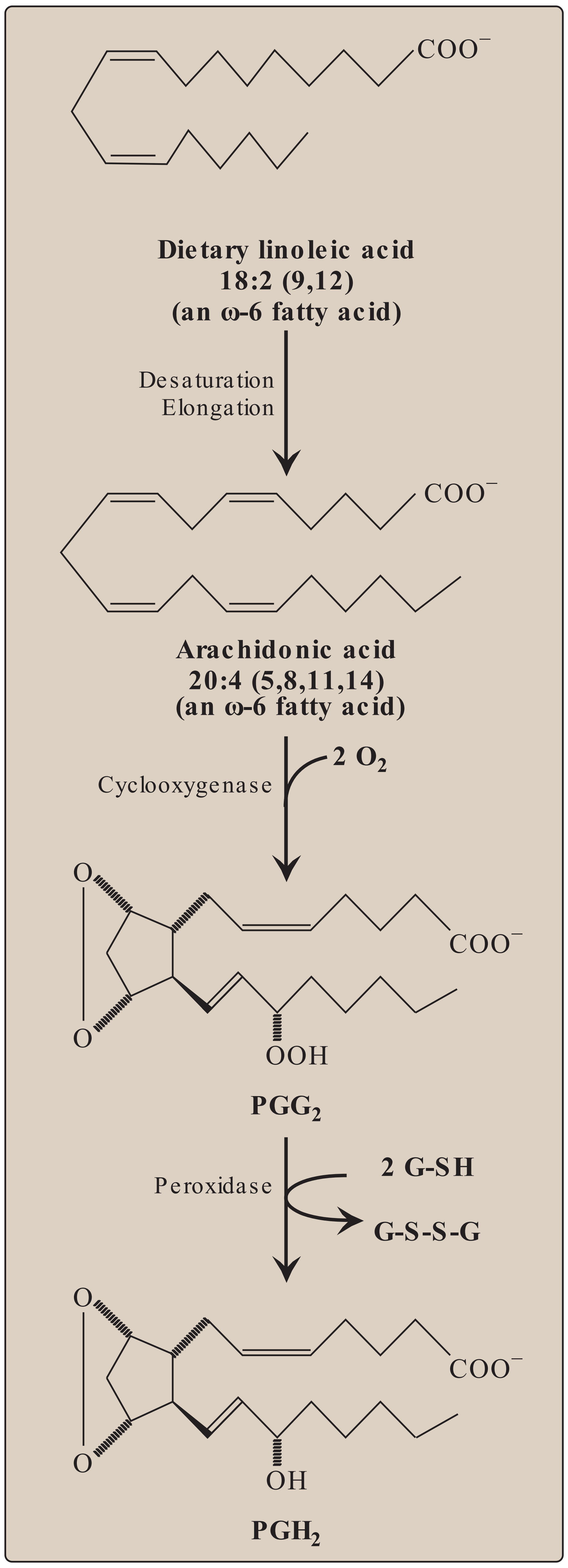


Figure 17.22
Oxidation and cyclization of arachidonic acid by the two catalytic activities (cyclooxygenase and peroxidase) of prostaglandin endoperoxide synthase. G-SH = reduced glutathione; G-S-S-G = oxidized glutathione.

2. Inhibition of prostaglandin synthesis: The synthesis of prostaglandins can be inhibited by a number of unrelated compounds. For example, cortisol (a steroidal anti-inflammatory agent) inhibits phospholipase A$_2$ activity (see Figure 17.23) and, therefore, the precursor of the prostaglandins, arachidonic acid, is not made available from membrane phospholipids. Aspirin, indomethacin, and phenylbutazone (all nonsteroidal anti-inflammatory drugs [NSAIDs]) inhibit both COX-1 and COX-2 and, thus, prevent the synthesis of the parent prostaglandin, PGH$_2$. [Note: Systemic inhibition of COX-1, with subsequent damage to the stomach and the kidneys and impaired clotting of blood, is the basis of aspirin's toxicity.] Aspirin (but not other NSAIDs) also induces synthesis of lipoxins and resolvins, lipid mediators with anti-inflammatory effects that are made from arachidonic acid and EPA, respectively. Inhibitors specific for COX-2 (the coxibs, for example, celecoxib) were designed to reduce pathologic inflammatory processes mediated by COX-2 while maintaining the physiologic functions of COX-1. However, their use has been associated with increased risk of heart attacks attacks, likely as a result of decreased PGI$_2$ synthesis (see below), and some have been withdrawn from the market.

B. Synthesis of leukotrienes

Arachidonic acid is converted to a variety of linear hydroperoxy (–OOH) acids by a separate pathway involving a family of lipoxygenases (LOXs). For example, 5-lipoxygenase converts arachidonic acid to 5-hydroperoxy-6,8,11,14 eicosatetraenoic acid ([5-HPETE], see Figure 17.23). 5-HPETE is converted to a series of leukotrienes containing four double bonds, the nature of the final products varying according to the tissue. Leukotrienes are mediators of allergic response and inflammation. Their synthesis is not inhibited by NSAIDs. [Note: Aspirin-induced asthma is a response to overproduction of leukotrienes with NSAID use.] Inhibitors of 5-lipoxygenase and leukotriene receptor antagonists are used in the treatment of asthma.

C. Role of prostaglandins in platelet homeostasis

Thromboxane A$_2$ (TXA$_2$) is produced by COX-1 in activated platelets. It promotes adherence and aggregation of circulating platelets and contraction of vascular smooth muscle, thereby promoting formation of blood clots (thrombi). (See online Chapter 34.) Prostacyclin (PGI$_2$), produced by COX-2 in vascular endothelial cells, inhibits platelet aggregation and stimulates vasodilation and, so, impedes thrombogenesis. The opposing effects of TXA$_2$ and PGI$_2$ limit thrombi formation to sites of vascular injury. [Note: Aspirin has an antithrombogenic effect. It inhibits TXA$_2$ synthesis by COX-1 in platelets and PGI$_2$ synthesis by COX-2 in endothelial cells through irreversible acetylation of these isozymes (Figure 17.24). The inhibition of COX-1 cannot be overcome in platelets, which lack nuclei. However, the inhibition of COX-2 can be overcome in endothelial cells, because they have a nucleus and, therefore, can generate more of the enzyme. This difference is the basis of low-dose aspirin therapy used to lower the risk of stroke and heart attacks by decreasing formation of thrombi.]

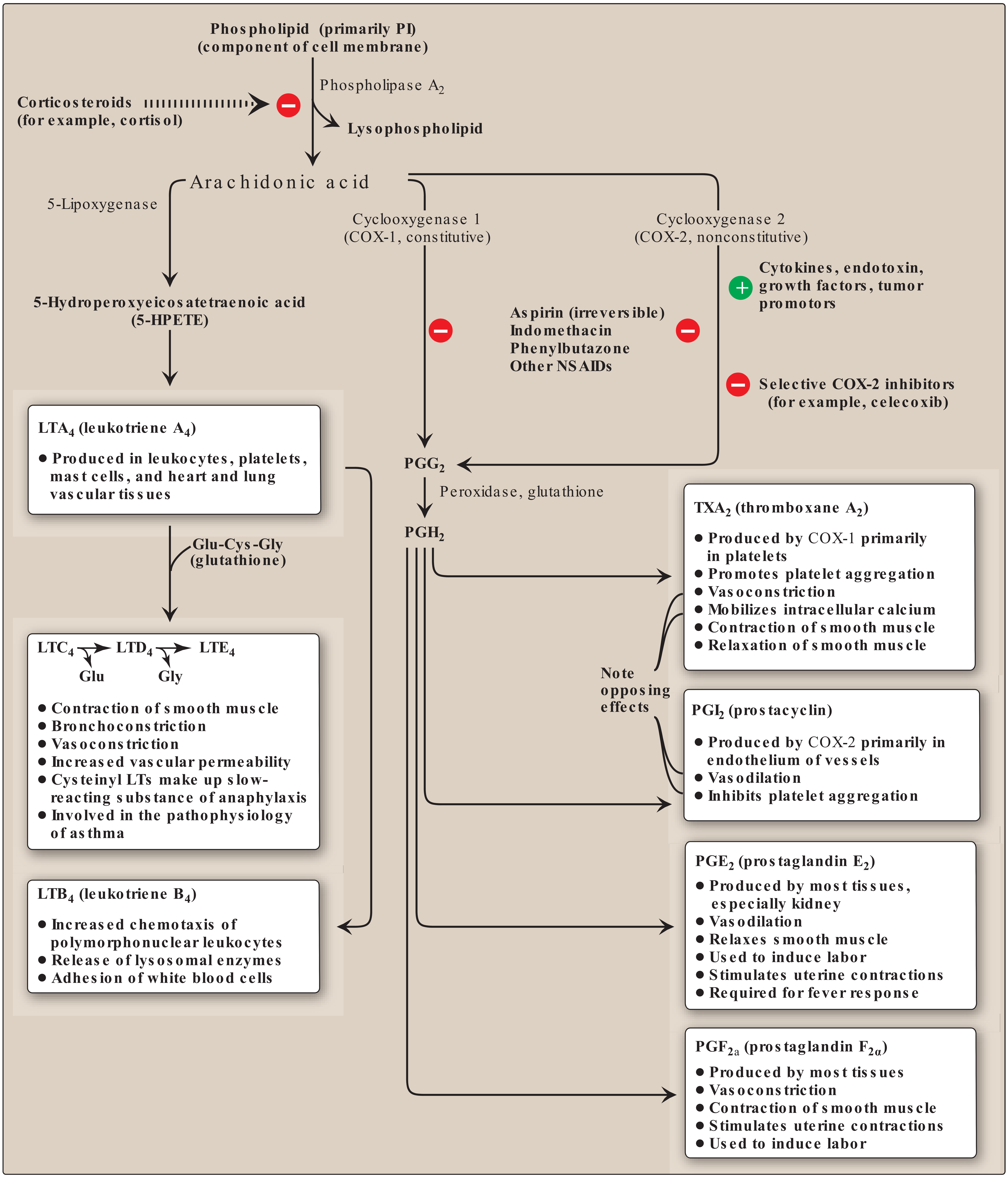

Phospholipid (primarily PI)
(component of cell membrane)
Phospholipase A_2
Corticosteroids
(for example, cortisol)
Lysophospholipid
Arachidonic acid
5-Lipoxygenase
Cyclooxygenase 1
(COX-1, constitutive)
Cyclooxygenase 2
(COX-2, nonconstitutive)
Cytokines, endotoxin, growth factors, tumor promotors
5-Hydroperoxyeicosatetraenoic acid
(5-HPETE)
Aspirin (irreversible)
Indomethacin
Phenylbutazone
Other NSAIDs
Selective COX-2 inhibitors
(for example, celecoxib)
LTA_4 (leukotriene A_4)
● Produced in leukocytes, platelets, mast cells, and heart and lung vascular tissues
PGG_2
Peroxidase, glutathione
PGH_2
TXA_2 (thromboxane A_2)
● Produced by COX-1 primarily in platelets
● Promotes platelet aggregation
● Vasoconstriction
● Mobilizes intracellular calcium
● Contraction of smooth muscle
● Relaxation of smooth muscle
Glu-Cys-Gly
(glutathione)
LTC_4
LTD_4
LTE_4
Glu
Gly
● Contraction of smooth muscle
● Bronchoconstriction
● Vasoconstriction
● Increased vascular permeability
● Cysteinyl LTs make up slow-reacting substance of anaphylaxis
● Involved in the pathophysiology of asthma
Note opposing effects
PGI_2 (prostacyclin)
● Produced by COX-2 primarily in endothelium of vessels
● Vasodilation
● Inhibits platelet aggregation
LTB_4 (leukotriene B_4)
● Increased chemotaxis of polymorphonuclear leukocytes
● Release of lysosomal enzymes
● Adhesion of white blood cells
PGE_2 (prostaglandin E_2)
● Produced by most tissues, especially kidney
● Vasodilation
● Relaxes smooth muscle
● Used to induce labor
● Stimulates uterine contractions
● Required for fever response
$PGF_{2\alpha}$ (prostaglandin $F_{2\alpha}$)
● Produced by most tissues
● Vasoconstriction
● Contraction of smooth muscle
● Stimulates uterine contractions
● Used to induce labor

Figure 17.23
Overview of the biosynthesis and function of some important prostaglandins (PGs), leukotrienes (LTs), and a thromboxane (TX) from arachidonic acid. [Note: The arachidonic acid in the membrane phospholipid was derived from the ω-6 essential fatty acid, linoleic, also an ω-6 fatty acid.] PI = phosphatidylinositol; NSAIDs = nonsteroidal anti-inflammatory drugs; Glu = glutamate; Cys = cysteine; Gly = glycine.

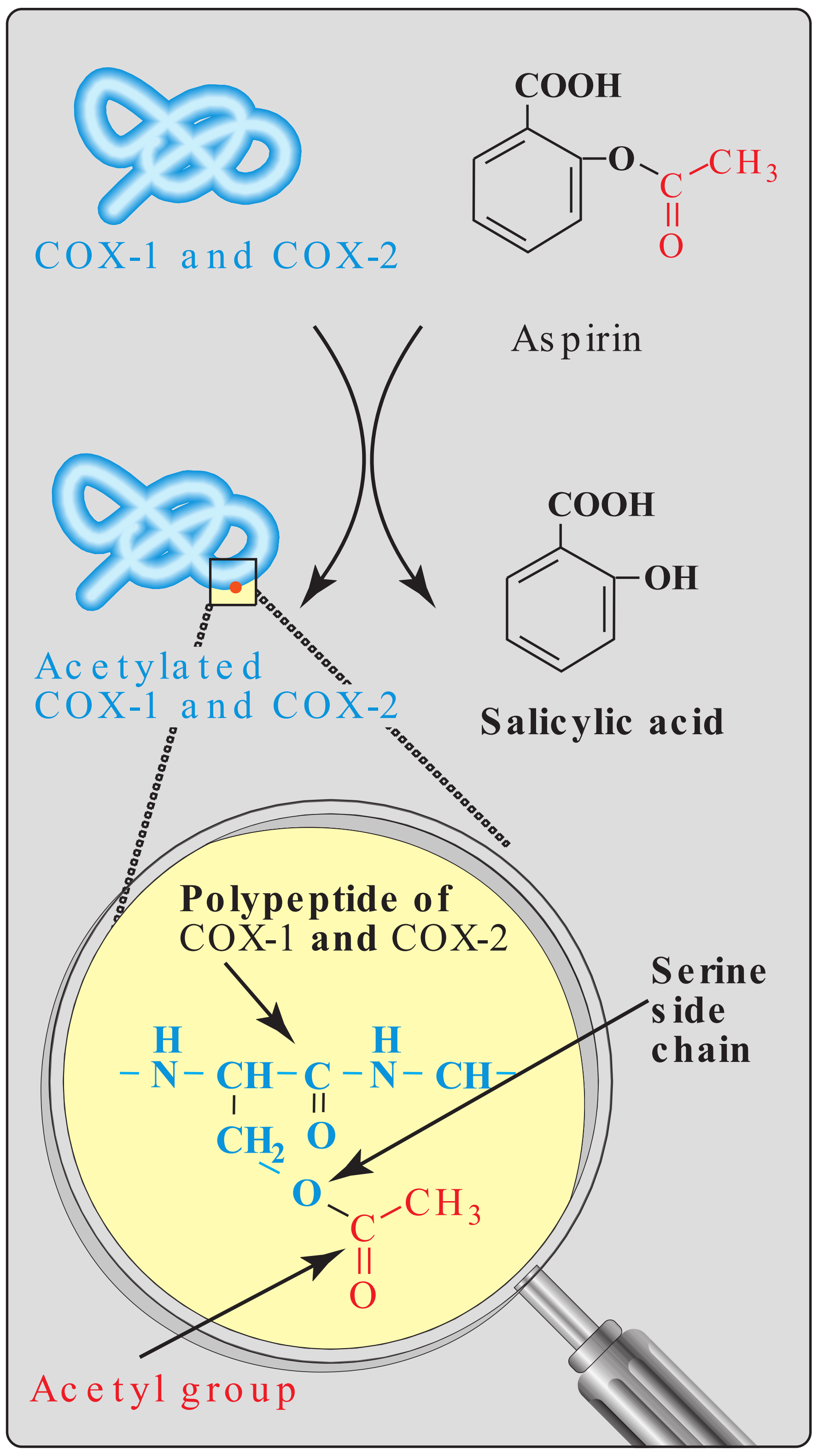


Figure 17.24
Irreversible acetylation of cyclooxygenase (COX)-1 and COX-2 by aspirin.

IX. CHAPTER SUMMARY

Phospholipids are **polar**, **ionic** compounds composed of an **alcohol** (for example, **choline** or **ethanolamine**) attached by a phosphodiester bond to either **diacylglycerol** (DAG), producing **phosphatidylcholine** or **phosphatidylethanolamine**, or to the amino alcohol **sphingosine** (Figure 17.25). Addition of a long-chain fatty acid to sphingosine produces a **ceramide**. Addition of a **phosphorylcholine** produces the phospholipid **sphingomyelin**. Phospholipids are the predominant lipids of **cell membranes**. Nonmembrane phospholipids serve as components of **lung surfactant** and **bile**. **Dipalmitoylphosphatidylcholine**, also called **dipalmitoyl lecithin**, is the major lipid component of **lung surfactant**. Insufficient surfactant production causes **respiratory distress syndrome**. **Phosphatidylinositol (PI)** serves as a reservoir for **arachidonic acid** in membranes. The phosphorylation of membrane-bound PI produces **phosphatidylinositol 4,5-bisphosphate** (**PIP_2**). This compound is degraded by phospholipase C in response to the binding of a variety of neurotransmitters, hormones, and growth factors to membrane G protein–coupled receptors. The products of this degradation, **inositol 1,4,5-trisphosphate** (**IP_3**) and **DAG** mediate the mobilization of intracellular **calcium** and the activation of protein kinase C, which act synergistically to evoke cellular responses. Specific proteins can be covalently attached via a carbohydrate bridge to membrane-bound phosphatidylinositol (**glycosyl phosphatidylinositol**, or **GPI**), forming a GPI anchor. A deficiency in the synthesis of GPI in hematopoietic cells results in a hemolytic disease, **paroxysmal nocturnal hemoglobinuria**. The **degradation** of phosphoglycerides is performed by phospholipases found in all tissues and pancreatic juice. **Sphingomyelin** is degraded to a ceramide plus phosphorylcholine by the lysosomal enzyme sphingomyelinase, a deficiency of which causes **Niemann-Pick (A + B) disease**. Glycosphingolipids are derivatives of **ceramides** to which carbohydrates have been attached. When one sugar molecule is added to the ceramide, a **cerebroside** is produced. If an oligosaccharide is added, a **globoside** is produced. If an acidic N-acetylneuraminic acid molecule is added, a **ganglioside** is produced. Glycosphingolipids are found predominantly in cell membranes of the **brain** and **peripheral nervous tissue**, with high concentrations in the **myelin sheath**. They are **antigenic**. Glycolipids are degraded in the **lysosomes** by acid hydrolases. A deficiency of any one of these enzymes produces a **sphingolipidosis**, in which a characteristic sphingolipid accumulates. **Prostaglandins** (**PGs**), **thromboxanes** (**TXs**), and **leukotrienes** (**LTs**) are produced in very small amounts in almost all tissues, act locally, and have an extremely short half-life. **They serve as mediators of the inflammatory response. Arachidonic acid** is the immediate precursor of the predominant class of PGs in humans (those with two double bonds). It is derived by the elongation and desaturation of the essential fatty acid linoleic acid and is stored in the membrane as a component of a phospholipid, generally PI. Arachidonic acid is released from the phospholipid by phospholipase A_2 (inhibited by cortisol). Synthesis of the **PGs** and **TXs** begins with the oxidative cyclization of free arachidonic acid to yield PGH_2 by prostaglandin endoperoxide synthase (PGH synthase), an endoplasmic reticulum membrane protein that has two catalytic activities: fatty acid cyclooxygenase (COX) and peroxidase. There are two isozymes of PGH synthase: COX-1 (constitutive) and COX-2 (nonconstitutive). Aspirin irreversibly inhibits both. Opposing effects of PGI_2 and TXA_2 limit clot formation. **LTs** are linear molecules produced from arachidonic acid by the 5-lipoxygenase pathway. They mediate allergic response and are not inhibited by aspirin or other NSAIDs.

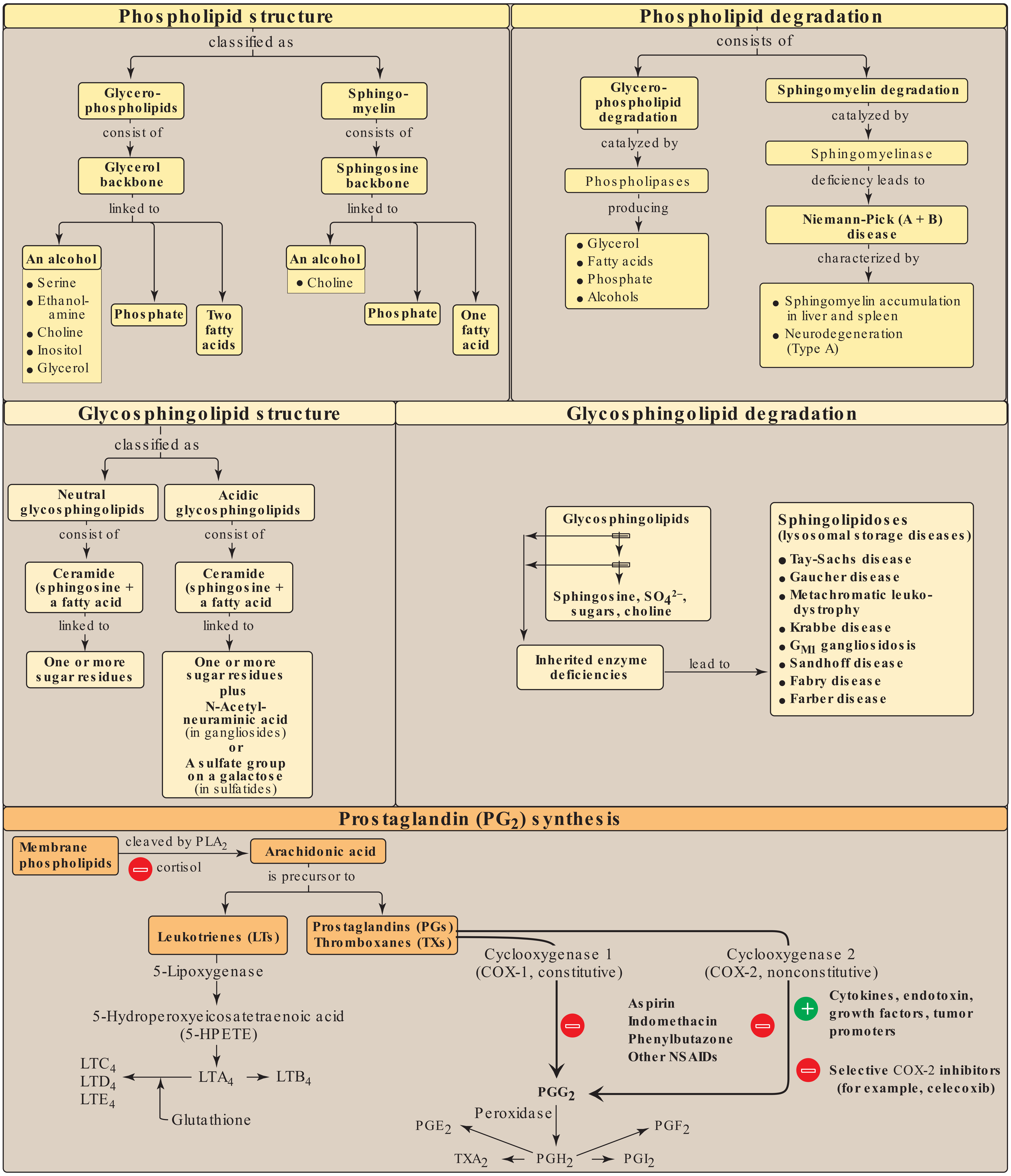

Phospholipid structure
classified as
Glycerophospholipids
consist of
Glycerol backbone
linked to
An alcohol
• Serine
• Ethanolamine
• Choline
• Inositol
• Glycerol
Phosphate
Two fatty acids
Sphingomyelin
consists of
Sphingosine backbone
linked to
An alcohol
• Choline
Phosphate
One fatty acid
Phospholipid degradation
consists of
Glycerophospholipid degradation
catalyzed by
Phospholipases
producing
• Glycerol
• Fatty acids
• Phosphate
• Alcohols
Sphingomyelin degradation
catalyzed by
Sphingomyelinase
deficiency leads to
Niemann-Pick (A + B) disease
characterized by
• Sphingomyelin accumulation in liver and spleen
• Neurodegeneration (Type A)
Glycosphingolipid structure
classified as
Neutral glycosphingolipids
consist of
Ceramide (sphingosine + a fatty acid
linked to
One or more sugar residues
Acidic glycosphingolipids
consist of
Ceramide (sphingosine + a fatty acid
linked to
One or more sugar residues plus N-Acetylneuraminic acid (in gangliosides) or A sulfate group on a galactose (in sulfatides)
Glycosphingolipid degradation
Glycosphingolipids
Sphingosine, SO_4^{2-}, sugars, choline
Inherited enzyme deficiencies
lead to
Sphingolipidoses (lysosomal storage diseases)
• Tay-Sachs disease
• Gaucher disease
• Metachromatic leukodystrophy
• Krabbe disease
• G_{M1} gangliosidosis
• Sandhoff disease
• Fabry disease
• Farber disease
Prostaglandin (PG_2) synthesis
Membrane phospholipids
cleaved by PLA_2
cortisol
Arachidonic acid
is precursor to
Leukotrienes (LTs)
5-Lipoxygenase
5-Hydroperoxyeicosatetraenoic acid (5-HPETE)
LTA_4
LTB_4
LTC_4
LTD_4
LTE_4
Glutathione
Prostaglandins (PGs) Thromboxanes (TXs)
Cyclooxygenase 1 (COX-1, constitutive)
Cyclooxygenase 2 (COX-2, nonconstitutive)
Aspirin
Indomethacin
Phenylbutazone
Other NSAIDs
Cytokines, endotoxin, growth factors, tumor promoters
Selective COX-2 inhibitors (for example, celecoxib)
PGG_2
Peroxidase
PGH_2
PGE_2
PGF_2
TXA_2
PGI_2

Figure 17.25
Key concept map for complex lipids. PLA_2 = phospholipase A_2; SO_4^{2-} = sulfate ion; NSAIDs = nonsteroidal anti-inflammatory drugs.

Cholesterol, Lipoprotein, and Steroid Metabolism

18

I. OVERVIEW

Cholesterol, the characteristic steroid alcohol of animal tissues, performs a number of essential functions in the body. For example, cholesterol is a structural component of all cell membranes, modulating their fluidity, and, in specialized tissues, cholesterol is a precursor of bile acids, steroid hormones, and vitamin D. It is, therefore, critically important that the cells of the body be assured an appropriate supply of cholesterol. To meet this need, a complex series of transport, biosynthetic, and regulatory mechanisms has evolved. The liver plays a central role in the regulation of the body's cholesterol homeostasis. For example, cholesterol enters the liver's cholesterol pool from a number of sources including dietary cholesterol as well as that synthesized de novo by extrahepatic tissues and by the liver itself. Cholesterol is eliminated from the liver as unmodified cholesterol in the bile, or it can be converted to bile salts that are secreted into the intestinal lumen. It can also serve as a component of plasma lipoproteins that carry lipids to the peripheral tissues. In humans, the balance between cholesterol influx and efflux is not precise, resulting in a gradual deposition of cholesterol in the tissues, particularly in the endothelial linings of blood vessels. This is a potentially life-threatening occurrence when the lipid deposition leads to plaque formation, causing the narrowing of blood vessels (atherosclerosis) and increased risk of cardio-, cerebro-, and peripheral vascular disease. Figure 18.1 summarizes the major sources of liver cholesterol and the routes by which cholesterol leaves the liver.

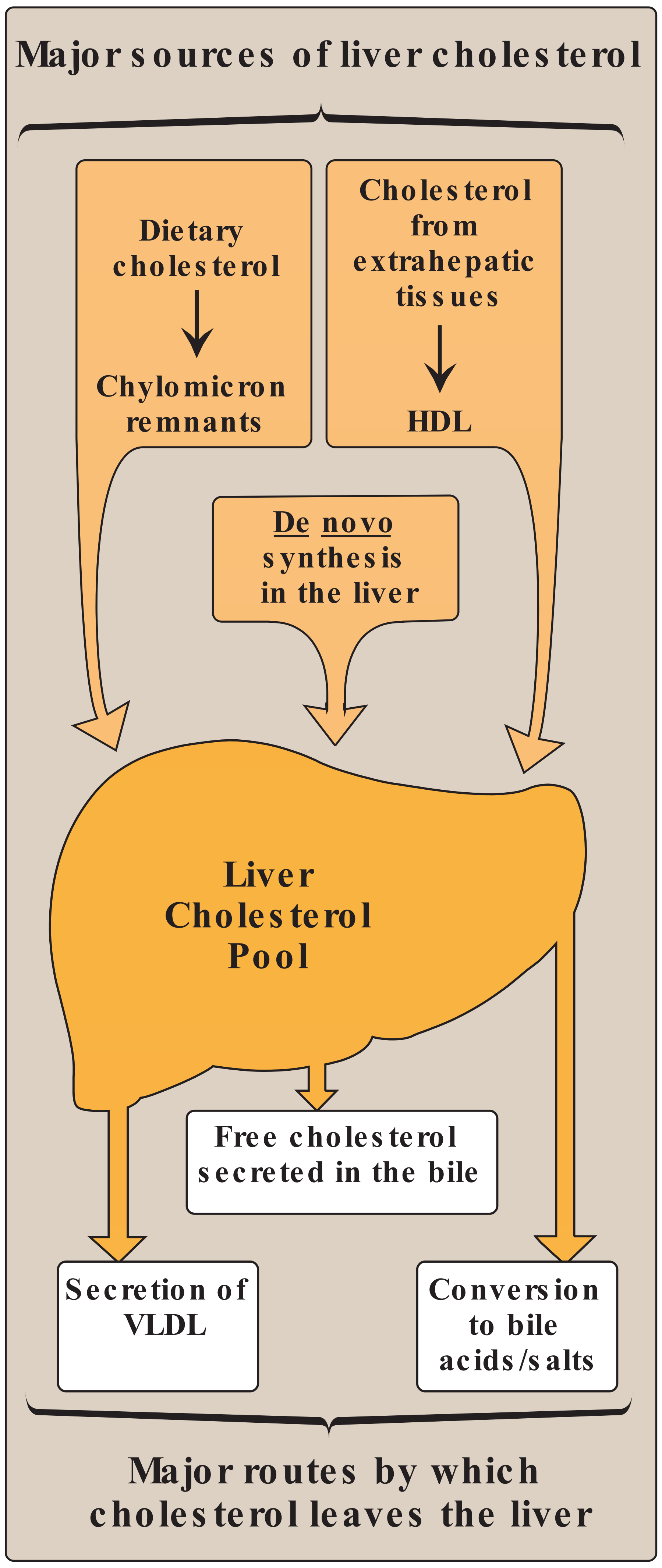

Major sources of liver cholesterol
Dietary cholesterol
Chylomicron remnants
Cholesterol from extrahepatic tissues
HDL
De novo synthesis in the liver
Liver Cholesterol Pool
Free cholesterol secreted in the bile
Secretion of VLDL
Conversion to bile acids/salts
Major routes by which cholesterol leaves the liver

Figure 18.1
Sources of liver cholesterol (influx) and routes by which cholesterol leaves the liver (efflux). HDL = high-density lipoprotein; VLDL = very-low-density lipoprotein.

II. STRUCTURE OF CHOLESTEROL

Cholesterol is a very hydrophobic compound. It consists of four fused hydrocarbon rings (A–D) called the "steroid nucleus," and it has an eight-carbon, branched hydrocarbon chain attached to carbon 17 of the D ring. Ring A has a hydroxyl group at carbon 3, and ring B has a double bond between carbon 5 and carbon 6 (Figure 18.2).

A. Sterols

Steroids with eight to ten carbon atoms in the side chain at carbon 17 and a hydroxyl group at carbon 3 are classified as sterols.

Cholesterol is the major sterol in animal tissues. It arises from de novo synthesis and absorption of dietary cholesterol. [Note: Intestinal uptake of cholesterol is mediated, at least in part, by the protein Niemann-Pick C1-like 1 protein (NPC1-L1), the target of the drug ezetimibe that reduces absorption of dietary cholesterol (see p. 176). Plant sterols (phytosterols), such as β-sitosterol, are poorly absorbed by humans (5% absorbed as compared to 40% for cholesterol). After entering the enterocytes, they are actively transported back into the intestinal lumen. Defects in the transporter result in the rare condition of sitosterolemia. Because some cholesterol is transported back as well, plant sterols reduce the absorption of dietary cholesterol. Daily ingestion of plant sterol esters supplied, for example, in spreads or juices, is one of a number of dietary strategies to reduce plasma cholesterol levels (see p. 363).]

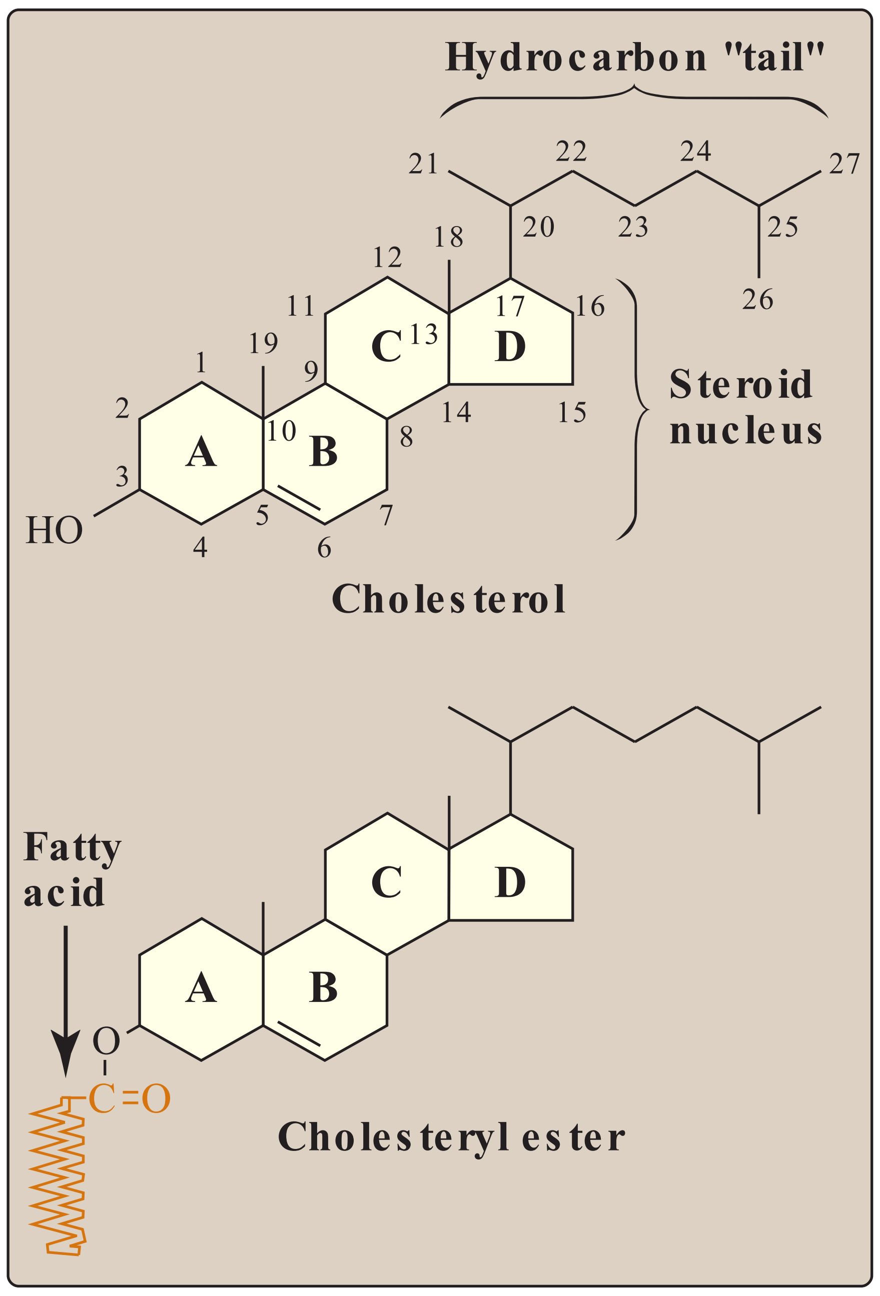


Figure 18.2
Structure of cholesterol and its ester.

B. Cholesteryl esters

Most plasma cholesterol is in an esterified form (with a fatty acid attached at carbon 3, as shown in Figure 18.2), which makes the structure even more hydrophobic than free (unesterified) cholesterol. Cholesteryl esters are not found in membranes and are normally present only in low levels in most cells. Because of their hydrophobicity, cholesterol and its esters must be transported in association with protein as a component of a lipoprotein particle (see p. 227) or be solubilized by phospholipids and bile salts in the bile (see p. 226).

III. SYNTHESIS OF CHOLESTEROL

Cholesterol is synthesized by virtually all tissues in humans, although liver, intestine, adrenal cortex, and reproductive tissues, including ovaries, testes, and placenta, make the largest contributions to the body's cholesterol pool. As with fatty acids, all the carbon atoms in cholesterol are provided by acetyl coenzyme A (CoA), and nicotinamide adenine dinucleotide phosphate (NADPH) provides the reducing equivalents. The pathway is endergonic, being driven by hydrolysis of the high-energy thioester bond of acetyl CoA and the terminal phosphate bond of adenosine triphosphate (ATP). Synthesis requires enzymes in both the cytosol and the membrane of the smooth endoplasmic reticulum (ER). The pathway is responsive to changes in cholesterol concentration, and regulatory mechanisms exist to balance the rate of cholesterol synthesis within the body against the rate of cholesterol excretion. An imbalance in this regulation can lead to an elevation in circulating levels of plasma cholesterol, with the potential for vascular disease.

A. Synthesis of 3-hydroxy-3-methylglutaryl coenzyme A

The first two reactions in the cholesterol synthetic pathway are similar to those in the pathway that produces ketone bodies (see Figure 16.22, p. 196). They result in the production of 3-hydroxy-3-methylglutaryl CoA ([HMG CoA] Figure 18.3). First, two acetyl CoA molecules condense to form acetoacetyl CoA. Next, a third molecule of acetyl CoA is added by HMG CoA synthase, producing HMG CoA, a six-carbon compound. [Note: Liver parenchymal cells contain two isoenzymes of the synthase. The cytosolic enzyme participates in cholesterol synthesis, whereas the mitochondrial enzyme functions in the pathway for ketone body synthesis.]

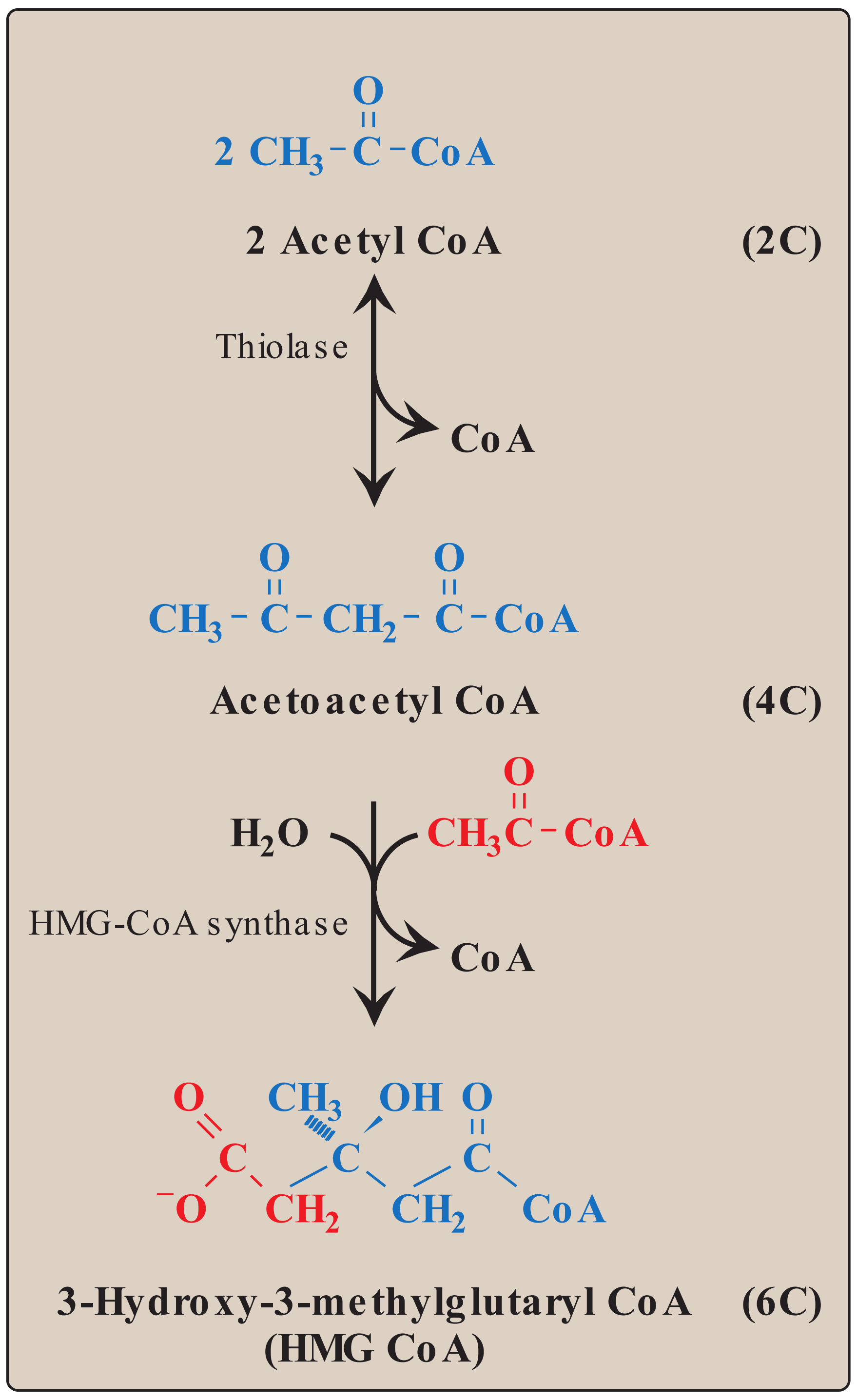


Figure 18.3
Synthesis of HMG CoA. CoA = coenzyme A.

B. Synthesis of mevalonate

The next step, the reduction of HMG CoA to mevalonate, is catalyzed by HMG CoA reductase and is the rate-limiting and key regulated step in cholesterol synthesis. It occurs in the cytosol, uses two molecules of NADPH as the reducing agent, and releases CoA, making the reaction irreversible (Figure 18.4). [Note: HMG CoA reductase is an integral membrane protein of the ER, with its catalytic domain projecting into the cytosol.] Regulation of reductase activity is discussed below.

C. Synthesis of cholesterol

The reactions and enzymes involved in the synthesis of cholesterol from mevalonate are illustrated in Figure 18.5. [Note: The numbers shown in brackets below correspond to numbered reactions shown in this figure.]

[1] Mevalonate is converted to 5-pyrophosphomevalonate in two steps, each of which transfers a phosphate group from ATP.

[2] A five-carbon isoprene unit, isopentenyl pyrophosphate (IPP), is formed by the decarboxylation of 5-pyrophosphomevalonate. The reaction requires ATP. [Note: IPP is the precursor of a family of molecules with diverse functions, the isoprenoids. Cholesterol is a sterol isoprenoid. Nonsterol isoprenoids include dolichol (see p. 167) and ubiquinone, or coenzyme Q (see p. 75).]

[3] IPP is isomerized to 3,3-dimethylallyl pyrophosphate (DPP).

[4] IPP and DPP condense to form ten-carbon geranyl pyrophosphate (GPP).

[5] A second molecule of IPP then condenses with GPP to form 15-carbon farnesyl pyrophosphate (FPP). [Note: Covalent attachment of farnesyl to proteins, a process known as "prenylation," is one mechanism for anchoring proteins (such as ras) to plasma membranes.]

[6] Two molecules of FPP combine, releasing pyrophosphate, and are reduced, forming the 30-carbon compound squalene. [Note: Squalene is formed from six isoprenoid units. Because three ATP are hydrolyzed per mevalonate residue converted to IPP, a total of 18 ATP are required to make the polyisoprenoid squalene.]

[7] Squalene is converted to the sterol lanosterol by a sequence of reactions catalyzed by ER-associated enzymes that use molecular oxygen and NADPH. The hydroxylation of linear squalene triggers the cyclization of the structure to lanosterol.

[8] The conversion of lanosterol to cholesterol is a multistep, ER-associated process involving shortening of the side-chain, oxidative removal of methyl groups, reduction of double bonds, and migration of a double bond. Smith-Lemli-Opitz syndrome (SLOS), an autosomal-recessive disorder of cholesterol biosynthesis, is caused by a partial deficiency in 7-dehydrocholesterol-7-reductase, the enzyme that reduces the double bond in 7-dehydrocholesterol (7-DHC), thereby converting it to cholesterol. SLOS is one of several multisystem, embryonic malformation syndromes associated with impaired cholesterol synthesis. [Note: 7-DHC is converted to vitamin D_3 in the skin (see p. 386).]

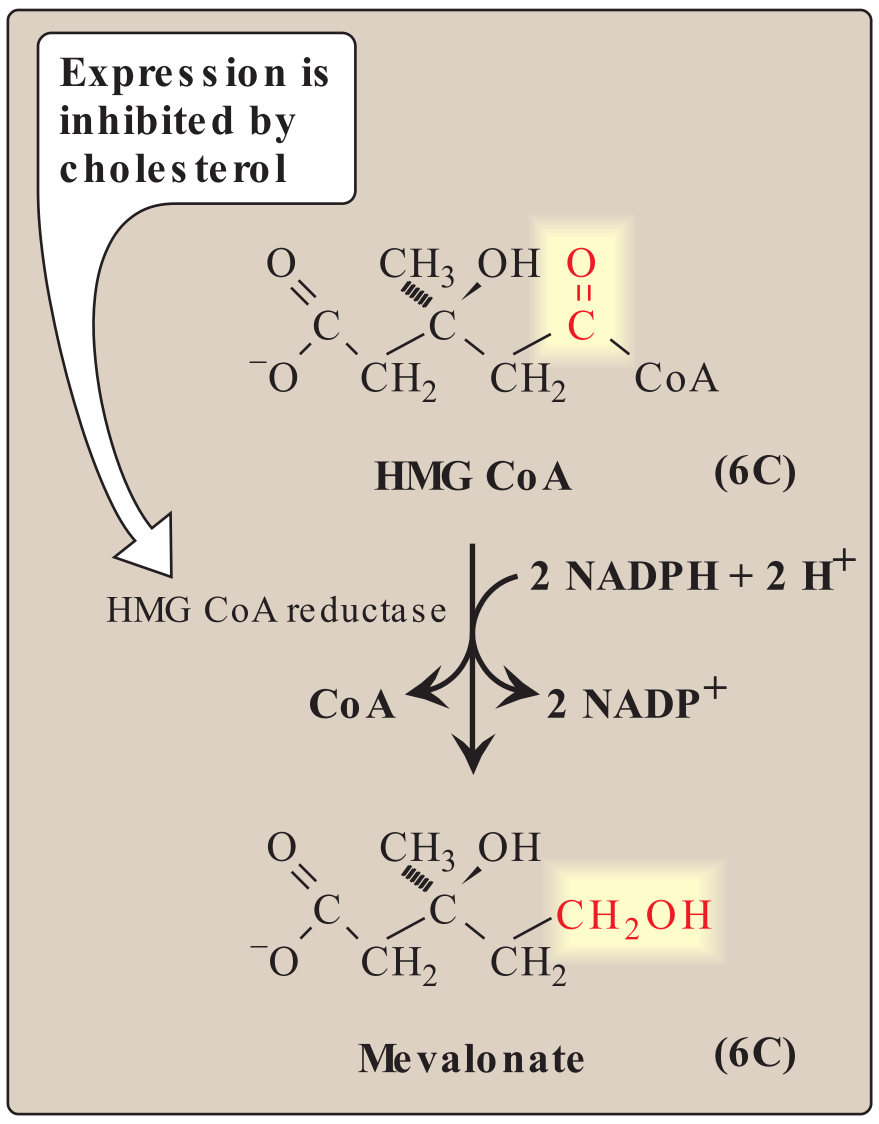
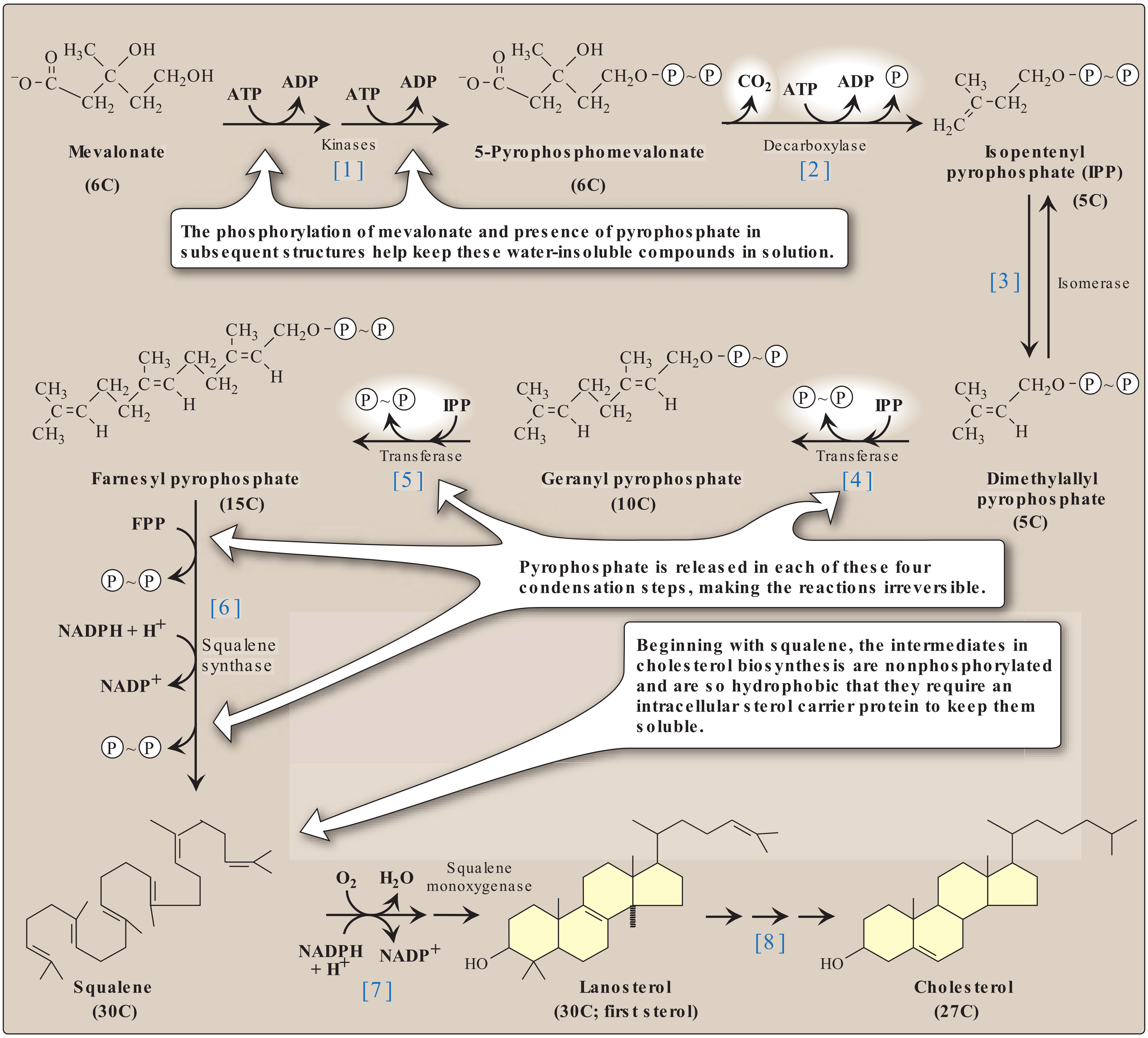


Figure 18.4
Synthesis of mevalonate. HMG CoA = hydroxymethylglutaryl coenzyme A; NADP(H) = nicotinamide adenine dinucleotide phosphate.


Mevalonate
(6C)
ATP
ADP
ATP
ADP
Kinases
[1]
5-Pyrophosphomevalonate
(6C)
CO_2
ATP
ADP
Ⓟ
Decarboxylase
[2]
Isopentenyl pyrophosphate (IPP)
(5C)
[3]
Isomerase
Dimethylallyl pyrophosphate
(5C)
Ⓟ~Ⓟ
IPP
Transferase
[4]
Geranyl pyrophosphate
(10C)
Ⓟ~Ⓟ
IPP
Transferase
[5]
Farnesyl pyrophosphate
(15C)
FPP
Ⓟ~Ⓟ
[6]
NADPH + H^+
Squalene synthase
$NADP^+$
Ⓟ~Ⓟ
Squalene
(30C)
O_2
H_2O
Squalene monoxygenase
NADPH + H^+
$NADP^+$
[7]
HO
Lanosterol
(30C; first sterol)
[8]
HO
Cholesterol
(27C)

The phosphorylation of mevalonate and presence of pyrophosphate in subsequent structures help keep these water-insoluble compounds in solution.

Pyrophosphate is released in each of these four condensation steps, making the reactions irreversible.

Beginning with squalene, the intermediates in cholesterol biosynthesis are nonphosphorylated and are so hydrophobic that they require an intracellular sterol carrier protein to keep them soluble.

Figure 18.5
Synthesis of cholesterol from mevalonate. ADP = adenosine diphosphate; Ⓟ = phosphate; Ⓟ~Ⓟ = pyrophosphate; NADP(H) = nicotinamide adenine dinucleotide phosphate.

D. Regulation of cholesterol synthesis

HMG CoA reductase is the major control point for cholesterol biosynthesis and is subject to different kinds of metabolic control.

1. **Sterol-dependent regulation of gene expression:** Expression of the gene for HMG CoA reductase is controlled by the transcription factor, SREBP-2 (sterol regulatory element–binding protein-2) that binds DNA at the cis-acting sterol regulatory element (SRE) upstream of the reductase gene. SREBP-2 is an integral protein of the ER membrane, and associates with a second ER membrane protein, SCAP (SREBP cleavage–activating protein). When sterol levels in the cell are low, the SREBP–SCAP complex moves from

the ER to the Golgi. In the Golgi membrane, SREBP-2 is sequentially acted upon by two proteases, which generate a soluble fragment that enters the nucleus, binds the SRE, and functions as a transcription factor. This results in increased synthesis of HMG CoA reductase and, therefore, increased cholesterol synthesis (Figure 18.6). If sterols are abundant, however, they bind SCAP at its sterol-sensing domain and induce the binding of SCAP to yet other ER membrane proteins, the insigs (insulin-induced gene [products]). This results in the retention of the SCAP–SREBP complex in the ER, thereby preventing the activation of SREBP-2, and leading to downregulation of cholesterol synthesis. [Note: SREBP-1 upregulates expression of enzymes involved in fatty acid synthesis in response to insulin (see p. 184).]

2. **Sterol-accelerated enzyme degradation:** The reductase itself is a sterol-sensing integral protein of the ER membrane. When sterol levels in the cell are high, the enzyme binds to insig proteins. Binding leads to ubiquitination and proteasomal degradation of the reductase (see p. 247).

3. **Sterol-independent phosphorylation/dephosphorylation:** HMG CoA reductase activity is controlled covalently through the actions of adenosine monophosphate (AMP)-activated protein kinase ([AMPK] see p. 183) and a phosphoprotein phosphatase (see Figure 18.6). The phosphorylated form of the enzyme is inactive, whereas the dephosphorylated form is active. [Note: Because AMPK is activated by AMP, cholesterol synthesis, like fatty acid synthesis, is decreased when ATP availability is decreased.]

4. **Hormonal regulation:** The amount of HMG CoA reductase is controlled hormonally. An increase in insulin and thyroxine favors upregulation of the expression of the gene for the reductase. Glucagon and the glucocorticoids have the opposite effect.

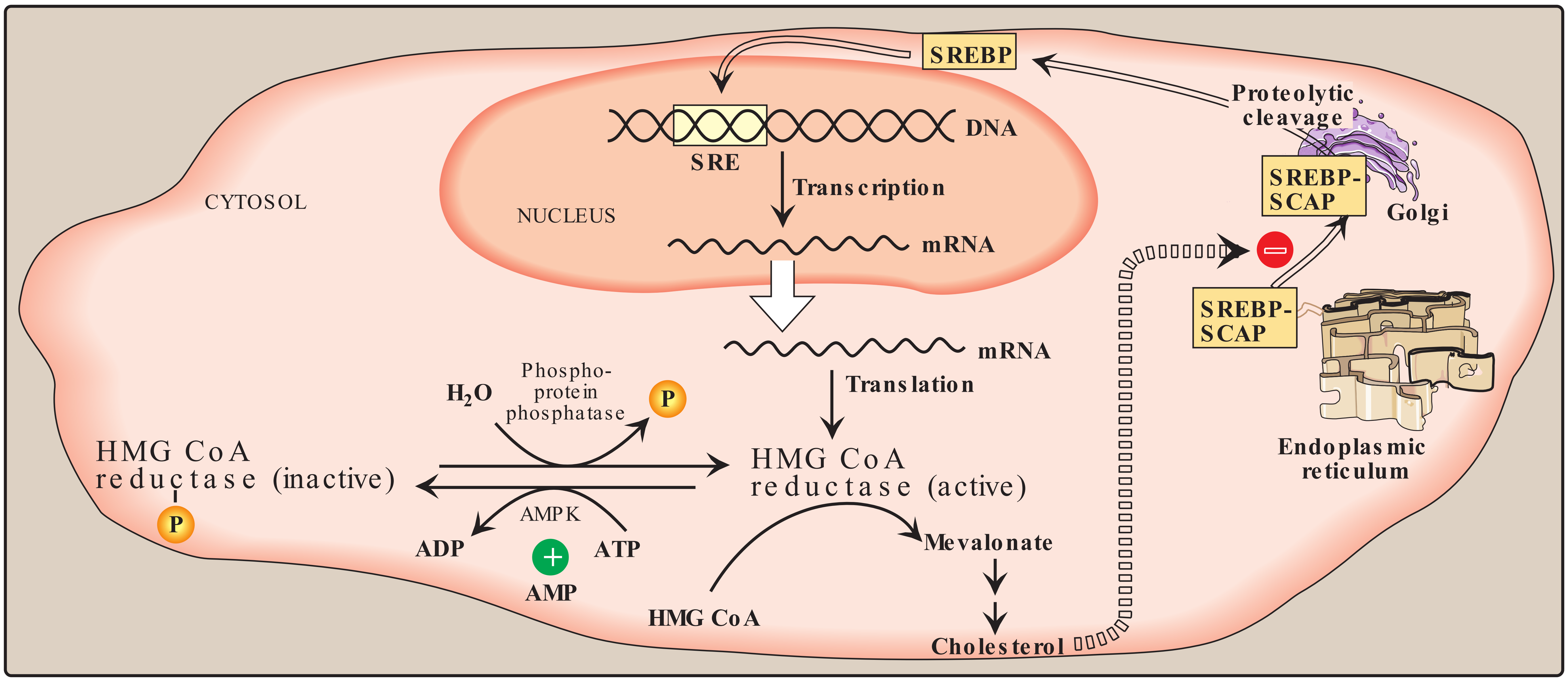

SREBP
DNA
SRE
Transcription
mRNA
CYTOSOL
NUCLEUS
Proteolytic cleavage
SREBP-SCAP
Golgi
SREBP-SCAP
Endoplasmic reticulum
mRNA
Translation
H_2O
Phosphoprotein phosphatase
P
HMG CoA reductase (inactive)
P
HMG CoA reductase (active)
AMPK
ADP
ATP
+
AMP
Mevalonate
HMG CoA
Cholesterol
−

Figure 18.6
Regulation of hydroxymethylglutaryl coenzyme A (HMG CoA) reductase. SRE = sterol regulatory element; SREBP = sterol regulatory element–binding protein; SCAP = SREBP cleavage–activating protein; AMPK = adenosine monophosphate–activated protein kinase; ADP = adenosine diphosphate; Ⓟ = phosphate; mRNA = messenger RNA.

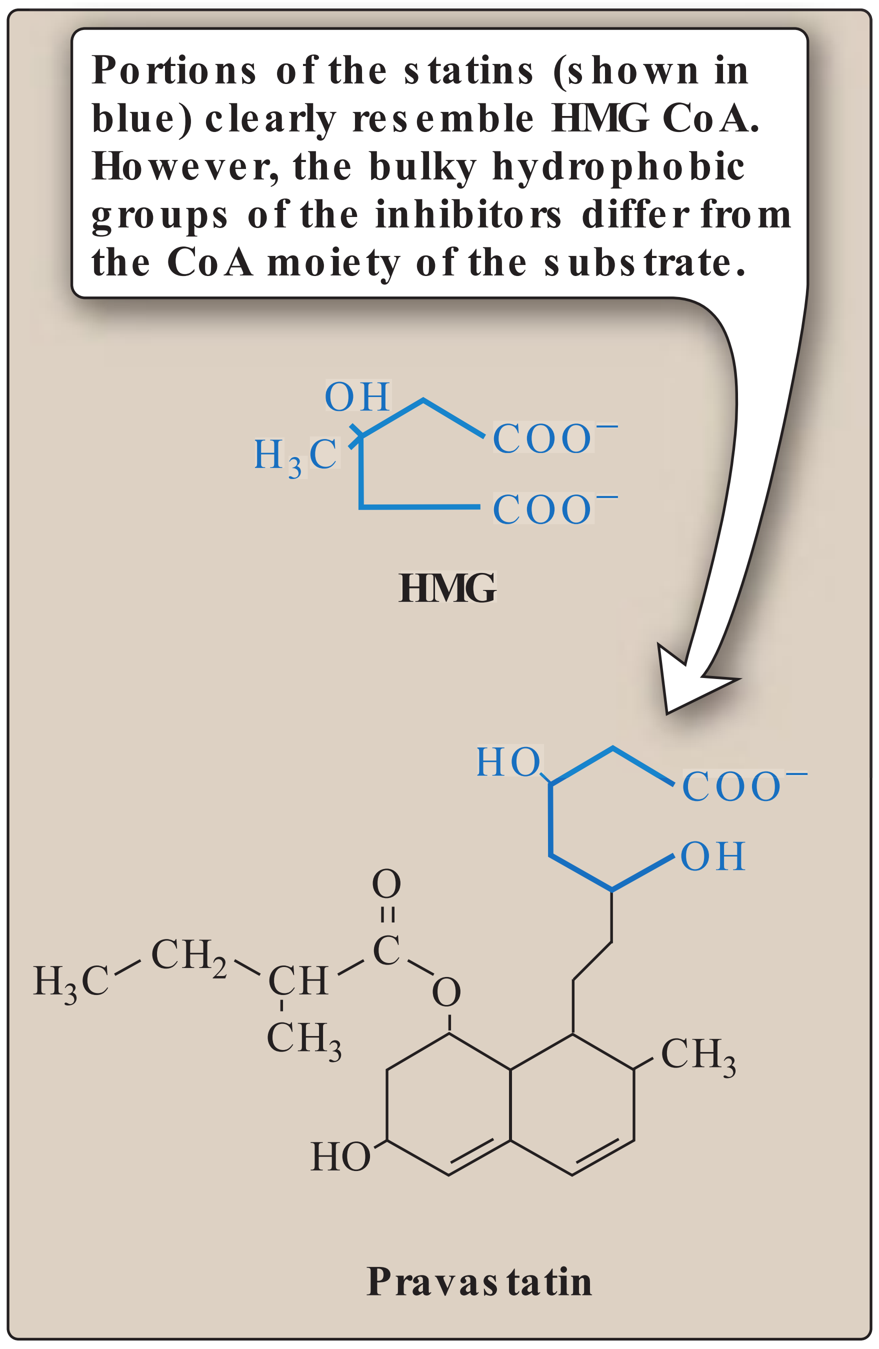

Portions of the statins (shown in blue) clearly resemble HMG CoA. However, the bulky hydrophobic groups of the inhibitors differ from the CoA moiety of the substrate.

Figure 18.7
Structural similarity of hydroxymethylglutaric acid (HMG) and pravastatin, a clinically useful cholesterol-lowering drug of the "statin" family. CoA = coenzyme A.

5. **Inhibition by drugs:** The statin drugs (atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin, and simvastatin) are structural analogs of HMG CoA, and are (or are metabolized to) reversible, competitive inhibitors of HMG CoA reductase (Figure 18.7). They are used to decrease plasma cholesterol levels in patients with hypercholesterolemia.

IV. DEGRADATION OF CHOLESTEROL

The ring structure of cholesterol cannot be metabolized to CO_2 and H_2O in humans. Rather, the intact sterol nucleus is eliminated from the body by conversion to bile acids and bile salts, a small percentage of which is excreted in the feces, and by secretion of cholesterol into the bile, which transports it to the intestine for elimination. Some of the cholesterol in the intestine is modified by bacteria before excretion. The primary compounds made are the isomers coprostanol and cholestanol, which are reduced derivatives of cholesterol. Together with cholesterol, these compounds make up the bulk of neutral fecal sterols.

V. BILE ACIDS AND BILE SALTS

Bile consists of a watery mixture of organic and inorganic compounds. Phosphatidylcholine, or lecithin (see p. 202), and conjugated bile salts are quantitatively the most important organic components of bile. Bile can either pass directly from the liver, where it is synthesized into the duodenum through the common bile duct, or be stored in the gallbladder when not immediately needed for digestion.

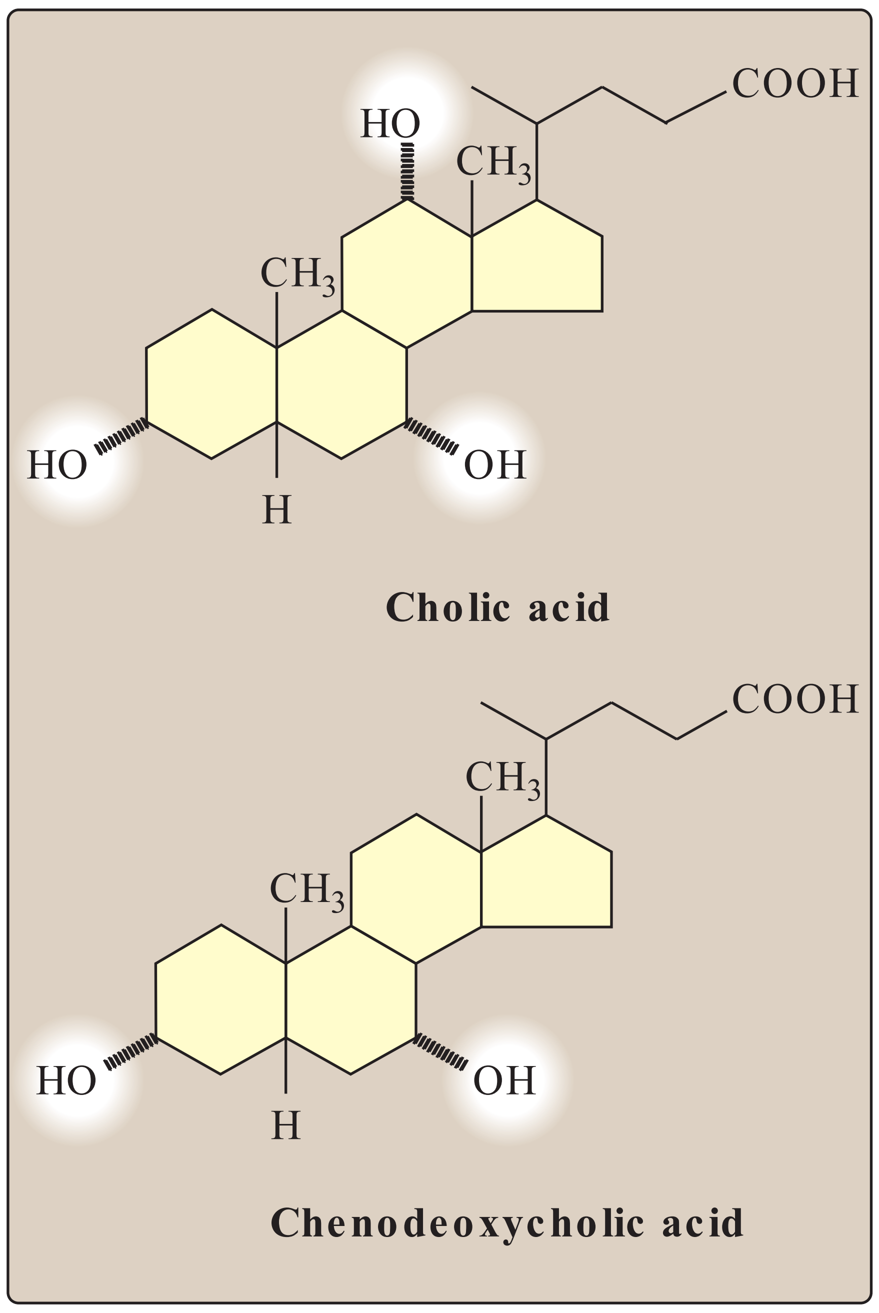


Figure 18.8
Bile acids. [Note: The ionized forms are bile salts.]

A. Structure of the bile acids

The bile acids contain 24 carbons, with two or three hydroxyl groups and a side chain that terminates in a carboxyl group. The carboxyl group has a pK_a of about 6. In the duodenum (pH approximately 6), this group will be protonated in half of the molecules (the bile acids) and deprotonated in the rest (the bile salts). The terms "bile acid" and "bile salt" are frequently used interchangeably, however. Both forms have hydroxyl groups that are α in orientation (they lie "below" the plane of the rings) and the methyl groups that are β (they lie "above" the plane of the rings). Therefore, the molecules have both a polar and a nonpolar face and can act as emulsifying agents in the intestine, helping prepare dietary triacylglycerol and other complex lipids for degradation by pancreatic digestive enzymes.

B. Synthesis of bile acids

Bile acids are synthesized in the liver by a multistep, multiorganelle pathway in which hydroxyl groups are inserted at specific positions on the steroid structure; the double bond of the cholesterol B ring is reduced; and the hydrocarbon chain is shortened by three carbons, introducing a carboxyl group at the end of the chain. The most common resulting compounds, cholic acid (a triol) and chenodeoxycholic acid (a diol), as shown in Figure 18.8, are called "primary" bile acids. [Note: The rate-limiting step in bile acid synthesis is the introduction of a hydroxyl group at carbon 7 of the steroid nucleus by 7-α-hydroxylase, an ER-associated cytochrome P450 monooxygen ase found only in liver. Expression of the enzyme is downregulated by bile acids (Figure 18.9)]

C. Synthesis of conjugated bile acids

Before the bile acids leave the liver, they are conjugated to a molecule of either glycine or taurine (an end product of cysteine metabolism) by an amide bond between the carboxyl group of the bile acid and the amino group of the added compound. These new structures include glycocholic and glycochenodeoxycholic acids and taurocholic and taurochenodeoxycholic acids (Figure 18.10). The ratio of glycine to taurine forms in the bile is approximately 3:1. Addition of glycine or taurine results in the presence of a carboxyl group with a lower pK_a (from glycine) or a sulfonate group (from taurine), both of which are fully ionized (negatively charged) at the alkaline pH of bile. The conjugated, ionized bile salts are more effective detergents than the unconjugated ones because of their enhanced amphipathic nature. Therefore, only the conjugated forms are found in the bile. Individuals with genetic deficiencies in the conversion of cholesterol to bile acids are treated with exogenously supplied chenodeoxycholic acid.

> Bile salts provide the only significant mechanism for cholesterol excretion, both as a metabolic product of cholesterol and as a solubilizer of cholesterol in bile.

D. Action of intestinal flora on bile salts

Bacteria in the intestine can deconjugate (remove glycine and taurine) bile salts. They can also remove the hydroxyl group at carbon 7, producing “secondary” bile salts such as deoxycholic acid from cholic acid and lithocholic acid from chenodeoxycholic acid (Figure 18.11).

E. Enterohepatic circulation

Bile salts secreted into the intestine are efficiently reabsorbed (greater than 95%) and reused. The liver actively secretes bile salts into the bile. In the intestine, they are reabsorbed in the terminal ileum via a Na^+-bile salt cotransporter and returned to the blood via a separate transport system. [Note: Lithocolic acid is only poorly absorbed.] They are efficiently taken up from blood by the hepatocytes via an isoform of the cotransporter and reused. [Note: Albumin binds bile salts noncovalently and transports them through the blood as was seen with fatty acids (see p. 181).] The continuous process of secretion of bile salts into the bile, their passage through the duodenum where some are deconjugated then dehydroxylated to secondary bile salts, their uptake in the ileum, and their subsequent return to the liver as a mixture of primary and secondary forms is termed the enterohepatic circulation (see Figure 18.11). Between 15 and 30 g of bile salts are secreted from the liver into the duodenum each day, yet only about 0.5 g (less than 3%) is lost daily in the feces. Approximately 0.5 g/day is synthesized from cholesterol in the liver to replace the amount lost. Bile acid sequestrants, such as cholestyramine, bind bile salts in the gut; prevent their reabsorption;

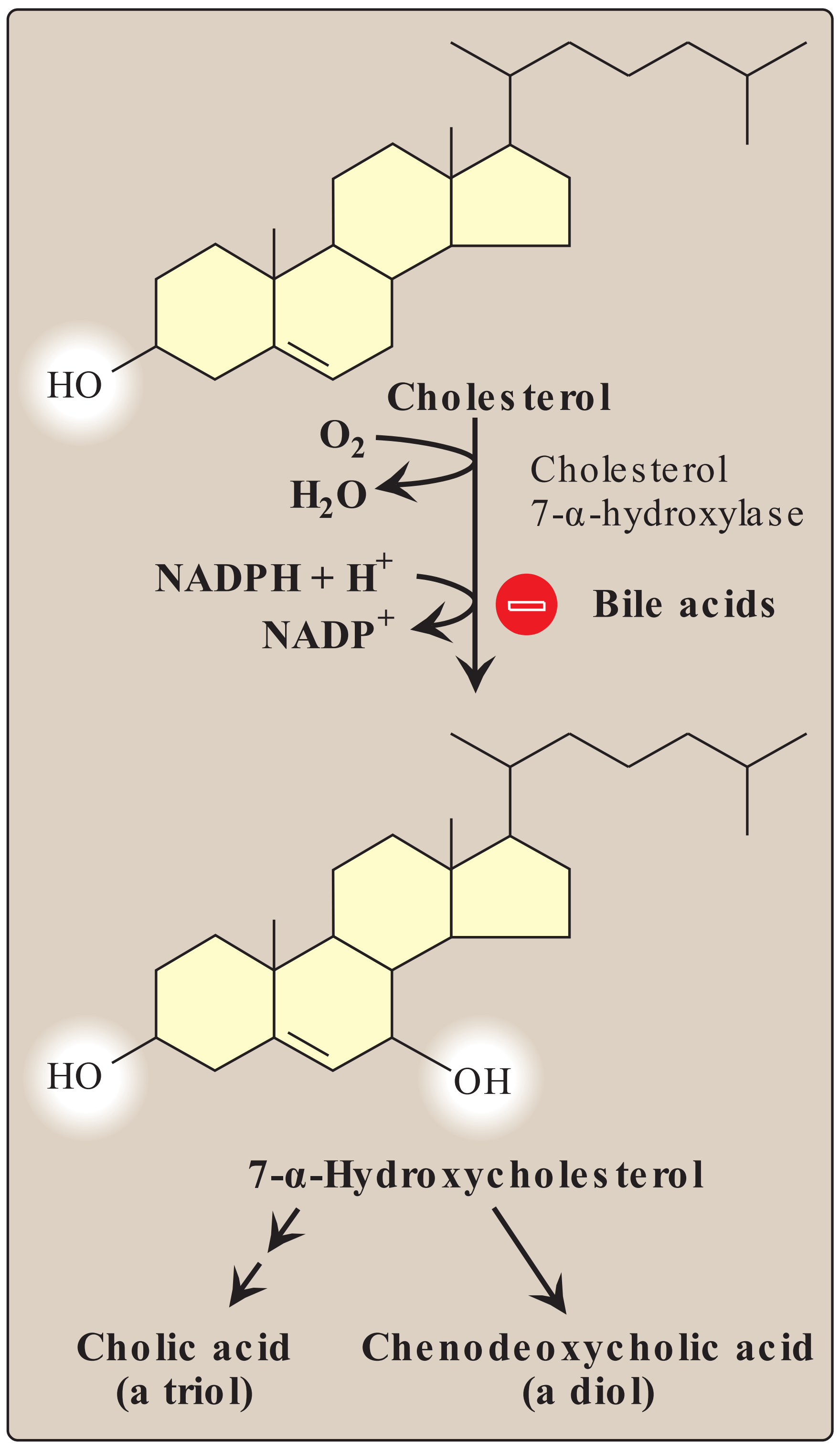

HO
Cholesterol
O_2
H_2O
Cholesterol 7-α-hydroxylase
$NADPH + H^+$
$NADP^+$
Bile acids
HO
OH
7-α-Hydroxycholesterol
Cholic acid (a triol)
Chenodeoxycholic acid (a diol)

Figure 18.9
Synthesis of the bile acids, cholic acid and chenodeoxycholic acid, from cholesterol.

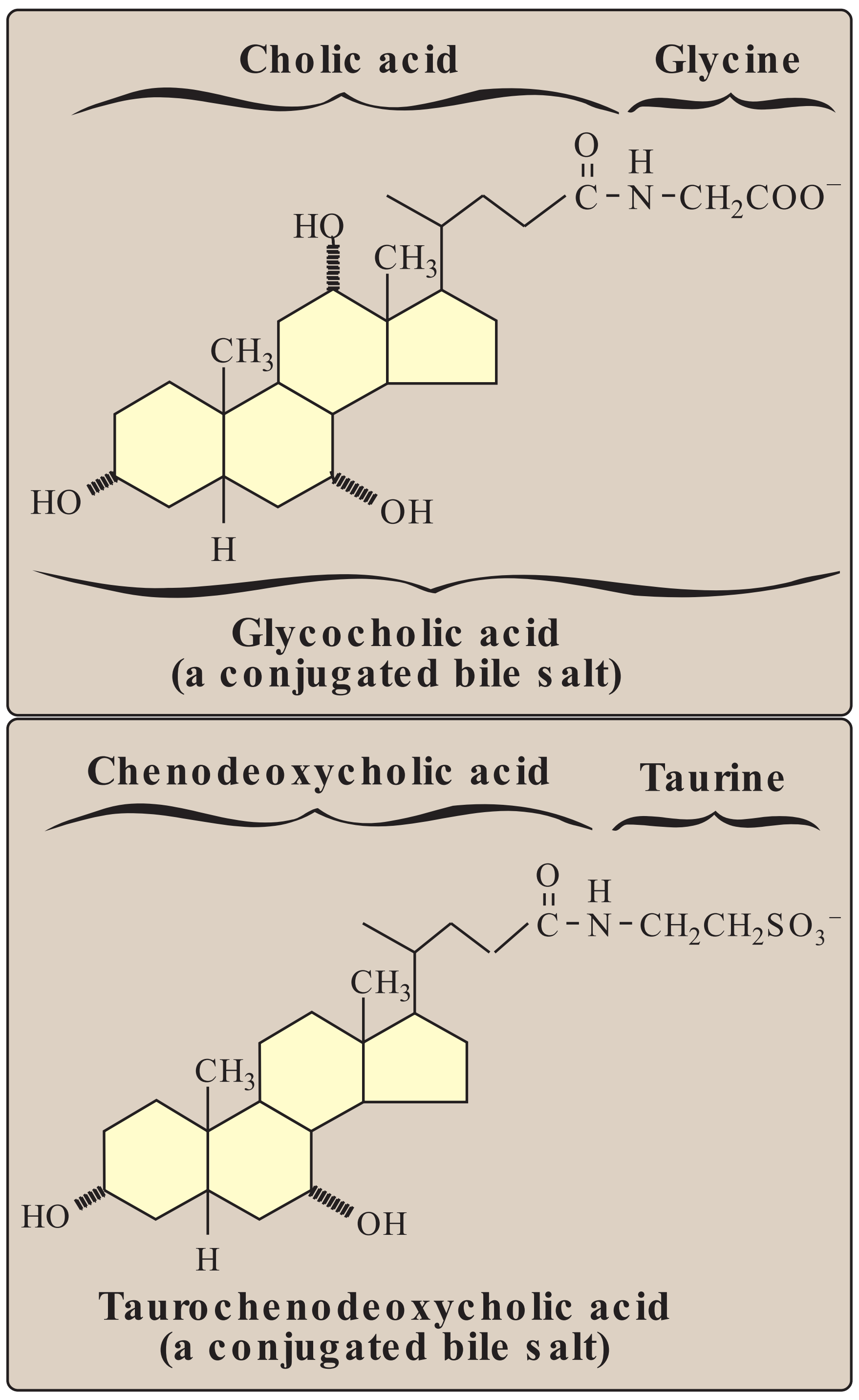

Cholic acid
Glycine
O
H
$C-N-CH_2COO^-$
HO
CH_3
CH_3
HO
OH
H
Glycocholic acid (a conjugated bile salt)
Chenodeoxycholic acid
Taurine
O
H
$C-N-CH_2CH_2SO_3^-$
CH_3
CH_3
HO
OH
H
Taurochenodeoxycholic acid (a conjugated bile salt)

Figure 18.10
Conjugated bile salts. Note “cholic” in the names.

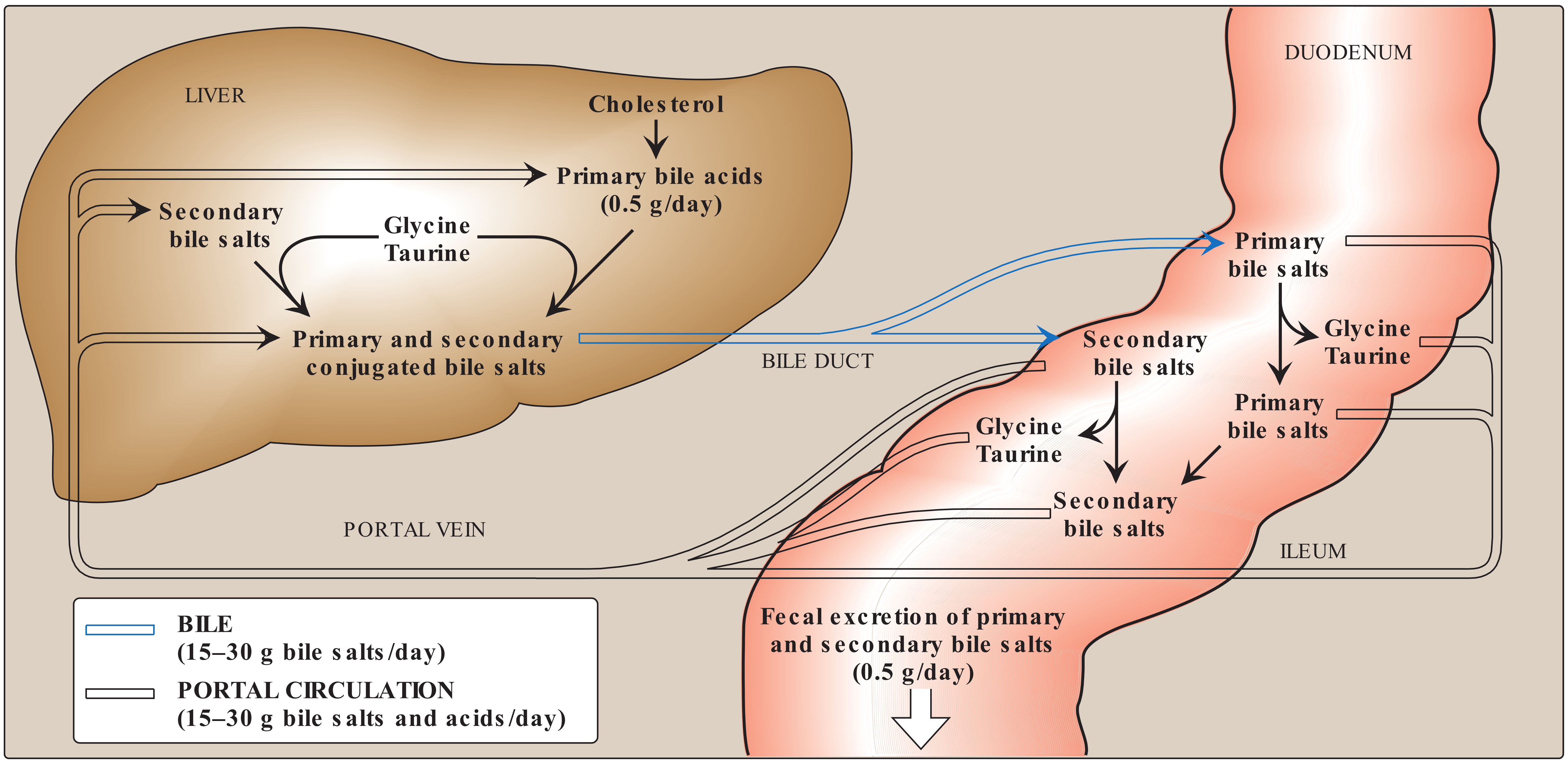

LIVER
Cholesterol
Primary bile acids (0.5 g/day)
Secondary bile salts
Glycine Taurine
Primary and secondary conjugated bile salts
BILE DUCT
PORTAL VEIN
DUODENUM
Primary bile salts
Glycine Taurine
Primary bile salts
Secondary bile salts
Glycine Taurine
Secondary bile salts
ILEUM
Fecal excretion of primary and secondary bile salts (0.5 g/day)
BILE (15–30 g bile salts/day)
PORTAL CIRCULATION (15–30 g bile salts and acids/day)

Figure 18.11
Enterohepatic circulation of bile salts. [Note: Primary forms are converted to secondary forms by dehydroxylation.]

and, so, promote their excretion. They are used in the treatment of hypercholesterolemia because the removal of bile salts relieves the inhibition on bile acid synthesis in the liver, thereby diverting additional cholesterol into that pathway. [Note: Dietary fiber also binds bile salts and increases their excretion (see p. 365).]

F. Bile salt deficiency: cholelithiasis

The movement of cholesterol from the liver into the bile must be accompanied by the simultaneous secretion of phospholipid and bile salts. If this dual process is disrupted and more cholesterol is present than can be solubilized by the bile salts and phosphatidylcholine present, the cholesterol may precipitate in the gallbladder, leading to cholesterol gallstone disease, or cholelithiasis (Figure 18.12). This disorder is typically caused by a decrease of bile acids in the bile. Cholelithiasis also may result from increased secretion of cholesterol into bile, as seen with the use of fibrates (for example, gemfibrozil) to reduce cholesterol (and triacylglycerol) in the blood. Laparoscopic cholecystectomy (surgical removal of the gallbladder through a small incision) is currently the treatment of choice. However, for patients who are unable to undergo surgery, oral administration of chenodeoxycholic acid to supplement the body's supply of bile acids results in a gradual (months to years) dissolution of the gallstones. [Note: Cholesterol stones account for over 85% of cases of cholelithiasis, with bilirubin and mixed stones accounting for the rest].

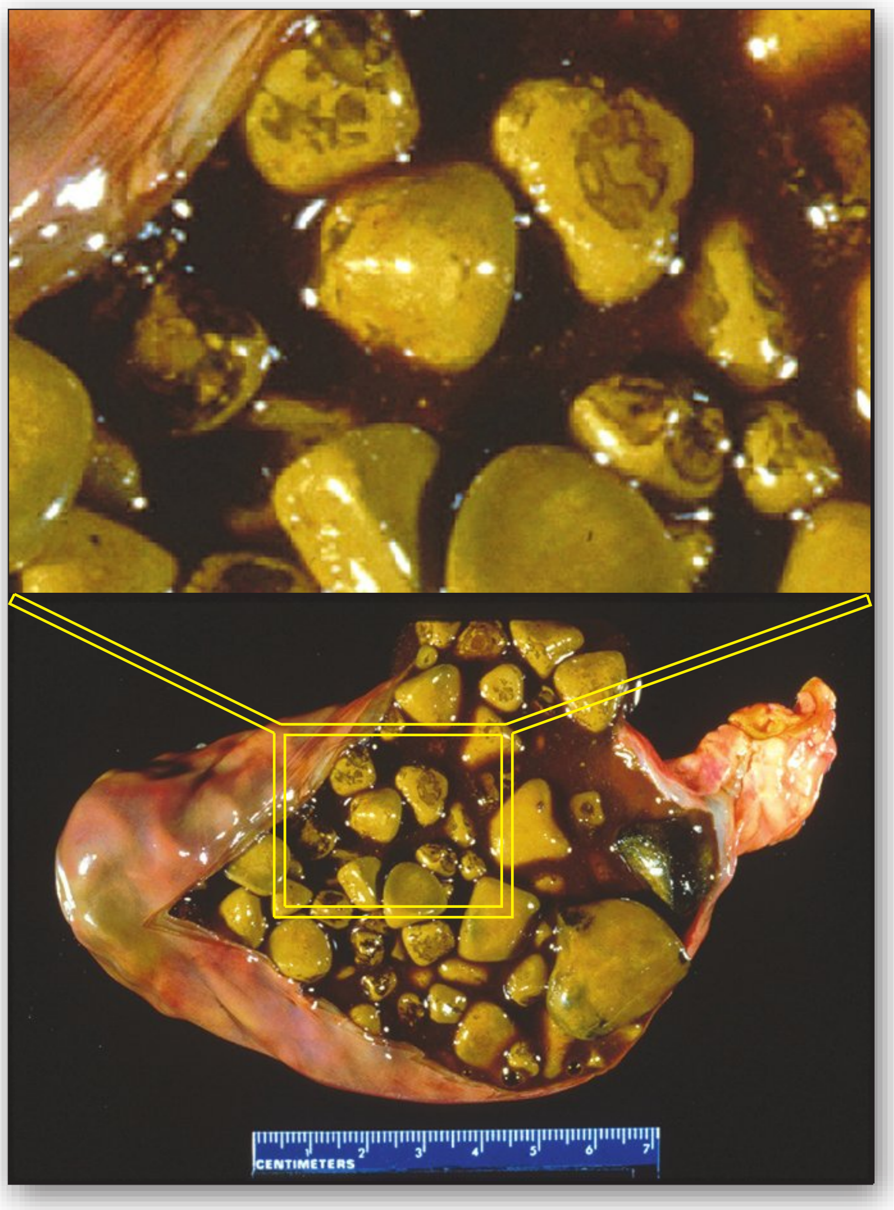

CENTIMETERS

Figure 18.12
Gallbladder with gallstones.

VI. PLASMA LIPOPROTEINS

The plasma lipoproteins are spherical macromolecular complexes of lipids and specific proteins (apolipoproteins). The lipoprotein particles include chylomicrons, very-low-density lipoproteins (VLDLs), low-density lipoproteins (LDLs), and high-density lipoproteins (HDLs). They differ in lipid and protein composition, size, density (Figure 18.13), and site of origin. [Note: Because lipoprotein particles constantly interchange lipids and apolipoproteins with each other, the actual apolipoprotein and lipid content of each class of particles is somewhat variable.] Lipoproteins function both to keep their component lipids soluble as they transport them in the plasma and to provide an efficient mechanism for transporting their lipid contents to (and from) the tissues. In humans, the transport system is less perfect than in other animals and, as a result, humans experience a gradual deposition of lipid (especially cholesterol) in tissues. This is a potentially life-threatening occurrence when the lipid deposition contributes to plaque formation, causing the narrowing of blood vessels (atherosclerosis).

A. Composition of plasma lipoproteins

Lipoproteins are composed of a neutral lipid core (containing triacylglycerol [TAG] and cholesteryl esters) surrounded by a shell of amphipathic apolipoproteins, phospholipid, and unesterified (free) cholesterol (Figure 18.14). These amphipathic compounds are oriented so that their polar portions are exposed on the surface of the lipoprotein, thereby rendering the particle soluble in aqueous solution. The TAG and cholesterol carried by the lipoproteins are obtained either from the diet (exogenous source) or from de novo synthesis (endogenous source). [Note: The cholesterol (C) content of plasma lipoproteins is now routinely measured in fasting blood. Total C = LDL – C + HDL – C + VLDL – C, where VLDL – C is calculated by dividing TAG by 5 because TAG represent 20% of the volume of VLDL. The goal value for total cholesterol is less than 200 mg/dl.]

1. **Size and density of lipoprotein particles:** Chylomicrons are the lipoprotein particles lowest in density and largest in size and that contain the highest percentage of lipid (as TAG) and the lowest percentage of protein. VLDLs and LDLs are successively denser, having higher ratios of protein to lipid. HDL particles are the smallest and densest. Plasma lipoproteins can be separated on the basis of their electrophoretic mobility, as shown in Figure 18.15, or on the basis of their density by ultracentrifugation.

2. **Apolipoproteins:** The apolipoproteins associated with lipoprotein particles have a number of diverse functions, such as providing recognition sites for cell-surface receptors and serving as activators or coenzymes for enzymes involved in lipoprotein metabolism. Some of the apolipoproteins are required as essential structural components of the particles and cannot be removed (in fact, the particles cannot be produced without them), whereas others are transferred freely between lipoproteins. Apolipoproteins are divided by structure and function into several major classes, denoted by letters, with each class having subclasses (for example, apolipoprotein [apo] C-I, apo C-II, and apo C-III). [Note: Functions of all of the apolipoproteins are not yet known.]

B. Metabolism of chylomicrons

Chylomicrons are assembled in intestinal mucosal cells and carry dietary (exogenous) TAG, cholesterol, fat-soluble vitamins, and

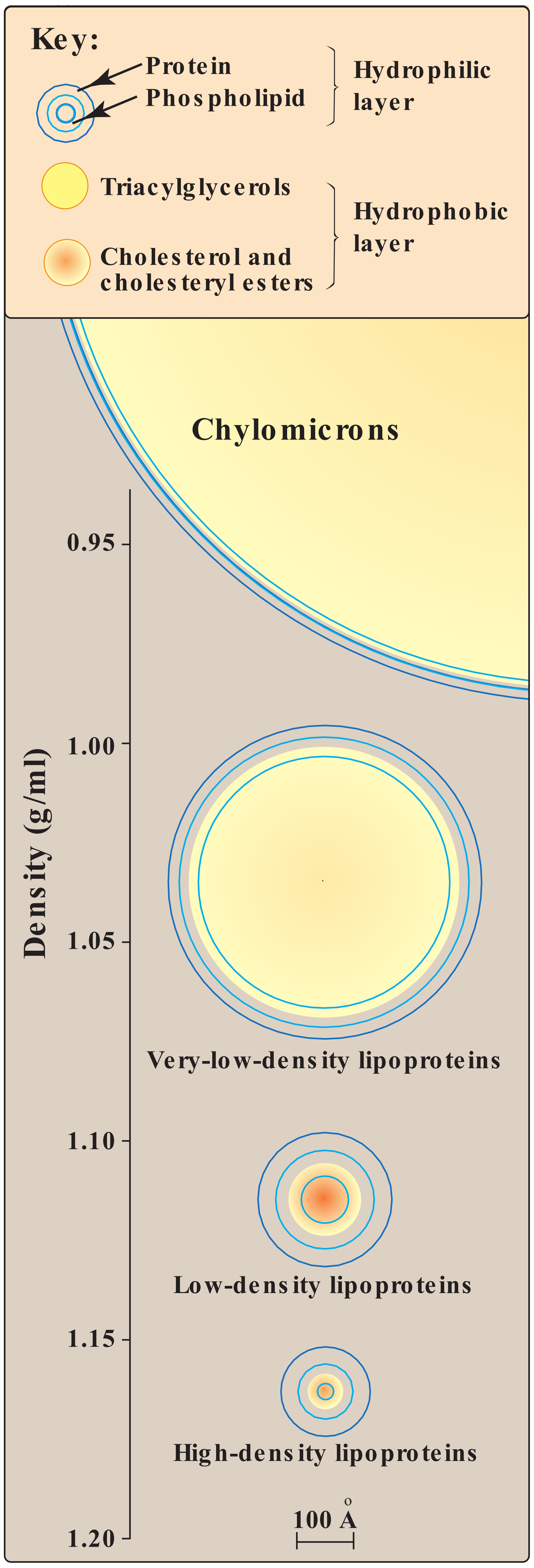


Figure 18.13
Approximate size and density of serum lipoproteins. Each family of lipoproteins exhibits a range of sizes and densities, and this figure shows typical values. The width of the rings approximates the amount of each component. [Note: Although cholesterol and its esters are shown as one component in the center of each particle, physically, cholesterol is a surface component, whereas cholesteryl esters are located in the interior of the lipoproteins.]

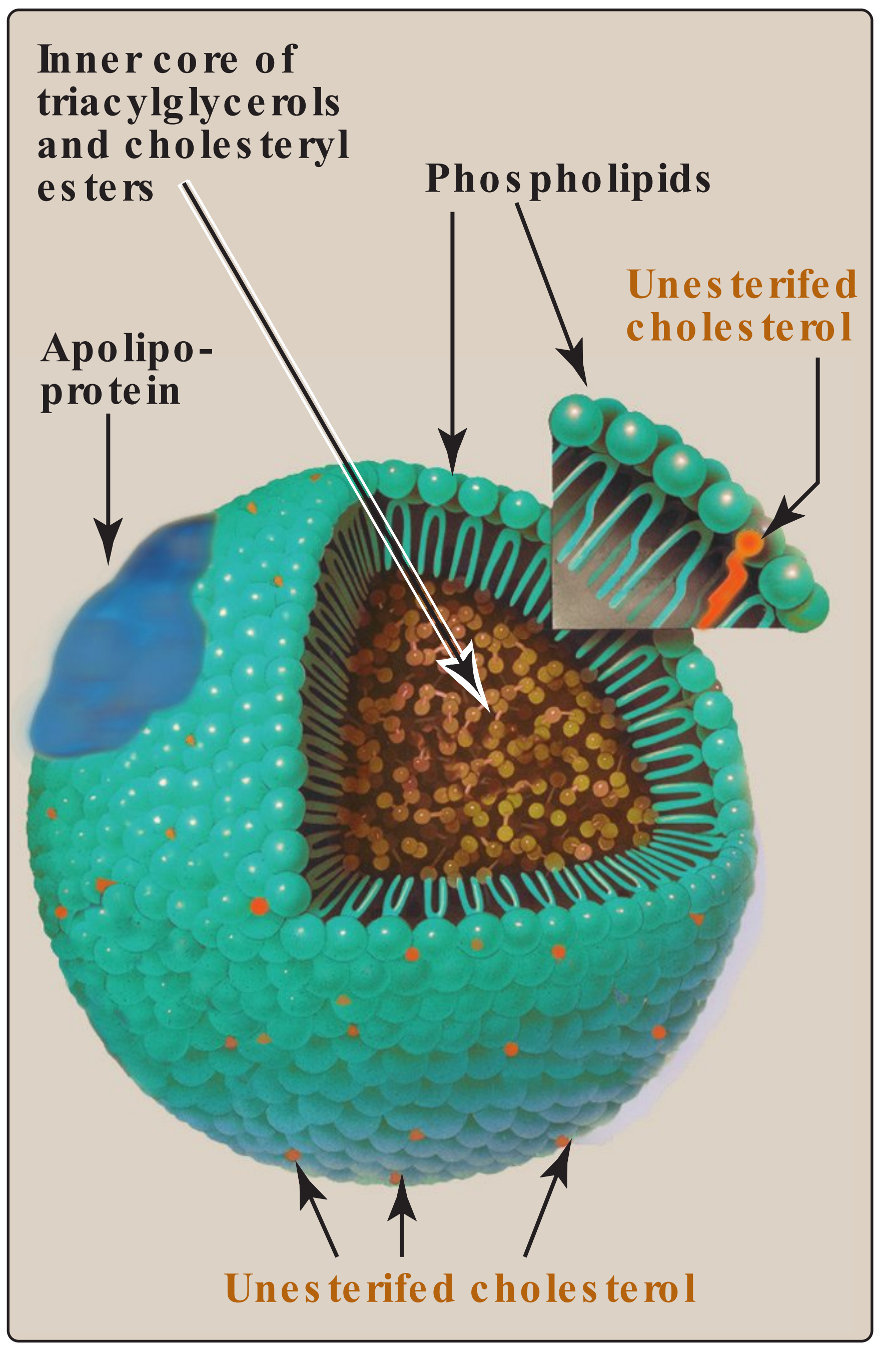


Figure 18.14
Structure of a typical lipoprotein particle.

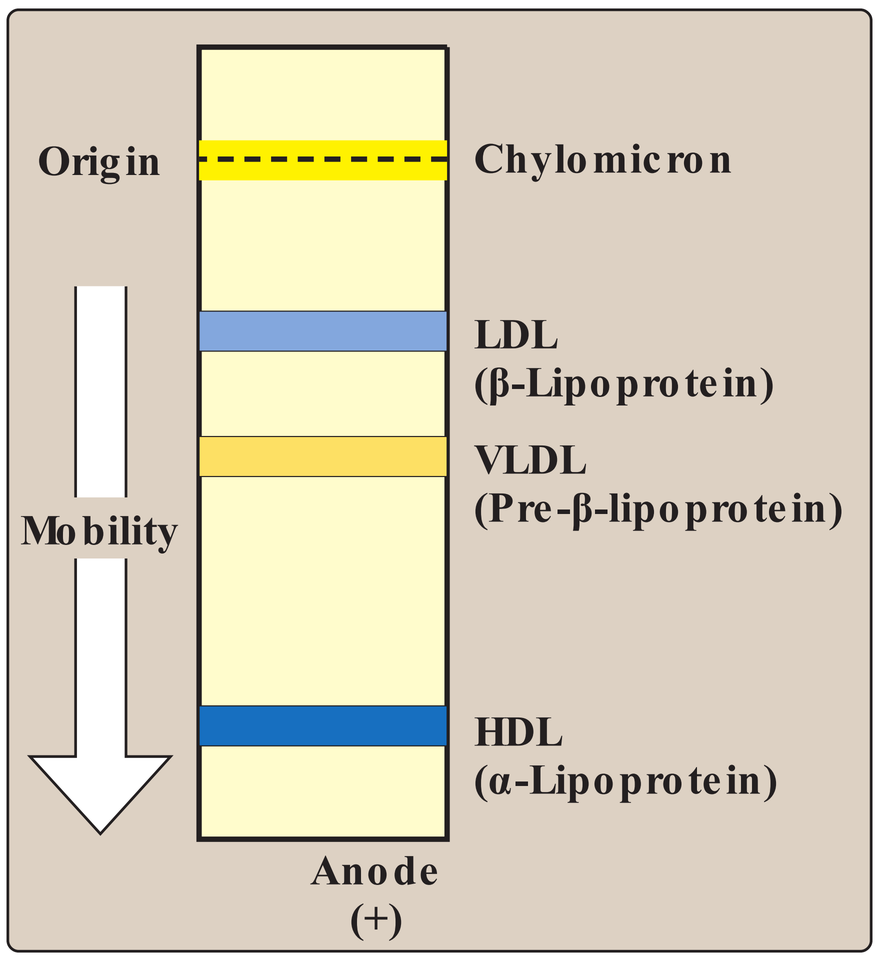


Figure 18.15
Electrophoretic mobility of plasma lipoproteins. The order of low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) is reversed if ultracentrifugation is used as the separation technique. HDL = high-density lipoprotein.

cholesteryl esters to the peripheral tissues (Figure 18.16). [Note: TAGs account for close to 90% of the lipids in a chylomicron.]

1. **Synthesis of apolipoproteins:** Apo B-48 is unique to chylomicrons. Its synthesis begins on the rough ER, and it is glycosylated as it moves through the ER and Golgi. [Note: Apo B-48 is so named because it constitutes the N-terminal 48% of the protein encoded by the gene for apo B. Apo B-100, which is synthesized by the liver and found in VLDL and LDL, represents the entire protein encoded by this gene. Posttranscriptional editing (see p. 457) of a cytosine to a uracil in intestinal apo B-100 messenger RNA (mRNA) creates a nonsense (stop) codon (see p. 433), allowing translation of only 48% of the mRNA.]

2. **Assembly of chylomicrons:** The enzymes involved in TAG, cholesterol, and phospholipid synthesis are located in the smooth ER. Assembly of the apolipoproteins and lipid into chylomicrons requires microsomal triglyceride transfer protein ([MTP], see p. 178), which loads apo B-48 with lipid. This occurs before transition from the ER to the Golgi, where the particles are packaged in secretory vesicles. These fuse with the plasma membrane releasing the lipoproteins, which then enter the lymphatic system and, ultimately, the blood. [Note: Chylomicrons leave the lymphatic system via the thoracic duct that empties into the left subclavian vein.]

3. **Modification of nascent chylomicron particles:** The particle released by the intestinal mucosal cell is called a "nascent" chylomicron because it is functionally incomplete. When it reaches the plasma, the particle is rapidly modified, receiving apolipoproteins E (which is recognized by hepatic receptors) and C. The latter includes apo C-II, which is necessary for the activation of lipoprotein lipase (LPL), the enzyme that degrades the TAG contained in the chylomicron (see below). The source of these apolipoproteins is circulating HDL (see Figure 18.16).

4. **Degradation of triacylglycerol by lipoprotein lipase:** LPL is an extracellular enzyme that is anchored by heparan sulfate to the capillary walls of most tissues, but predominantly those of adipose tissue and cardiac and skeletal muscle. Adult liver does not have this enzyme. [Note: A hepatic lipase is found on the surface of endothelial cells of the liver. It plays some role in TAG degradation in chylomirons and VLDL and is particularly important in HDL metabolism (see p. 236).] LPL, activated by apo C-II on circulating lipoprotein particles, hydrolyzes the TAG contained in these particles to yield fatty acids and glycerol. The fatty acids are stored (by the adipose) or used for energy (by the muscle). If they are not immediately taken up by a cell, the long-chain fatty acids are transported by serum albumin until their uptake does occur. The glycerol is used by the liver, for example, in lipid synthesis or gluconeogenesis. [Note: Patients with a deficiency of LPL or apo C-II (type 1 hyperlipoproteinemia, or familial LPL-deficiency) show a dramatic accumulation (1,000 mg/dl or greater) of chylomicron-TAG in the plasma (hypertriacylglycerolemia) even in the fasted state. These individuals are at increased risk for acute pancreatitis.]

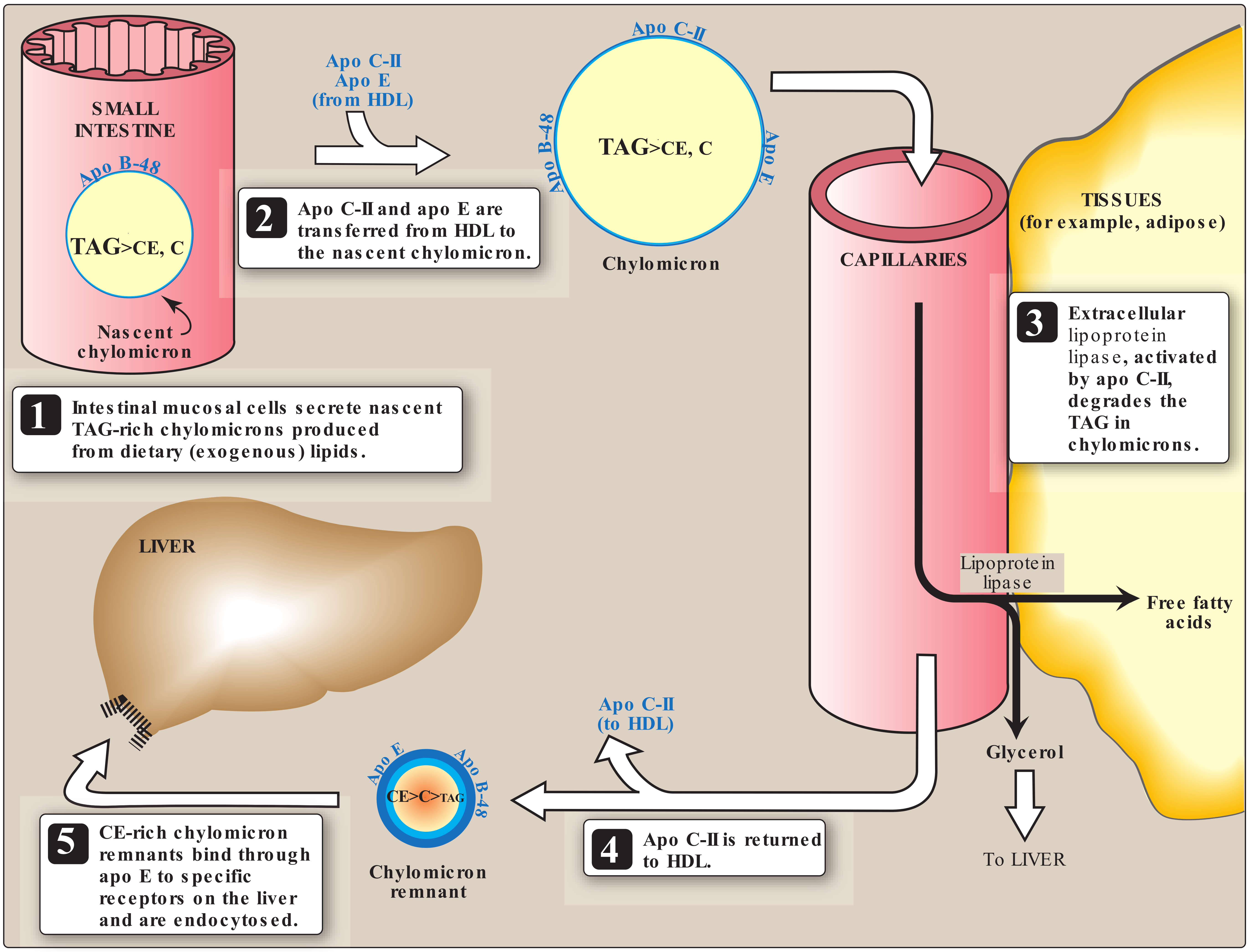

SMALL INTESTINE
Apo B-48
TAG>CE, C
Nascent chylomicron
Apo C-II
Apo E
(from HDL)
2 Apo C-II and apo E are transferred from HDL to the nascent chylomicron.
Apo C-II
Apo B-48
Apo E
TAG>CE, C
Chylomicron
CAPILLARIES
TISSUES
(for example, adipose)
1 Intestinal mucosal cells secrete nascent TAG-rich chylomicrons produced from dietary (exogenous) lipids.
3 Extracellular lipoprotein lipase, activated by apo C-II, degrades the TAG in chylomicrons.
LIVER
Lipoprotein lipase
Free fatty acids
Apo C-II
(to HDL)
Glycerol
Apo E
Apo B-48
CE>C>TAG
To LIVER
5 CE-rich chylomicron remnants bind through apo E to specific receptors on the liver and are endocytosed.
Chylomicron remnant
4 Apo C-II is returned to HDL.

Figure 18.16
Metabolism of chylomicrons. Apo B-48, apo C-II, and apo E are apolipoproteins found as specific components of plasma lipoproteins. The lipoprotein particles are not drawn to scale (see Figure 18.13 for details of their size and density). TAG = triacylglycerol; C = cholesterol; CE = cholesteryl ester; HDL = high-density lipoprotein particle.

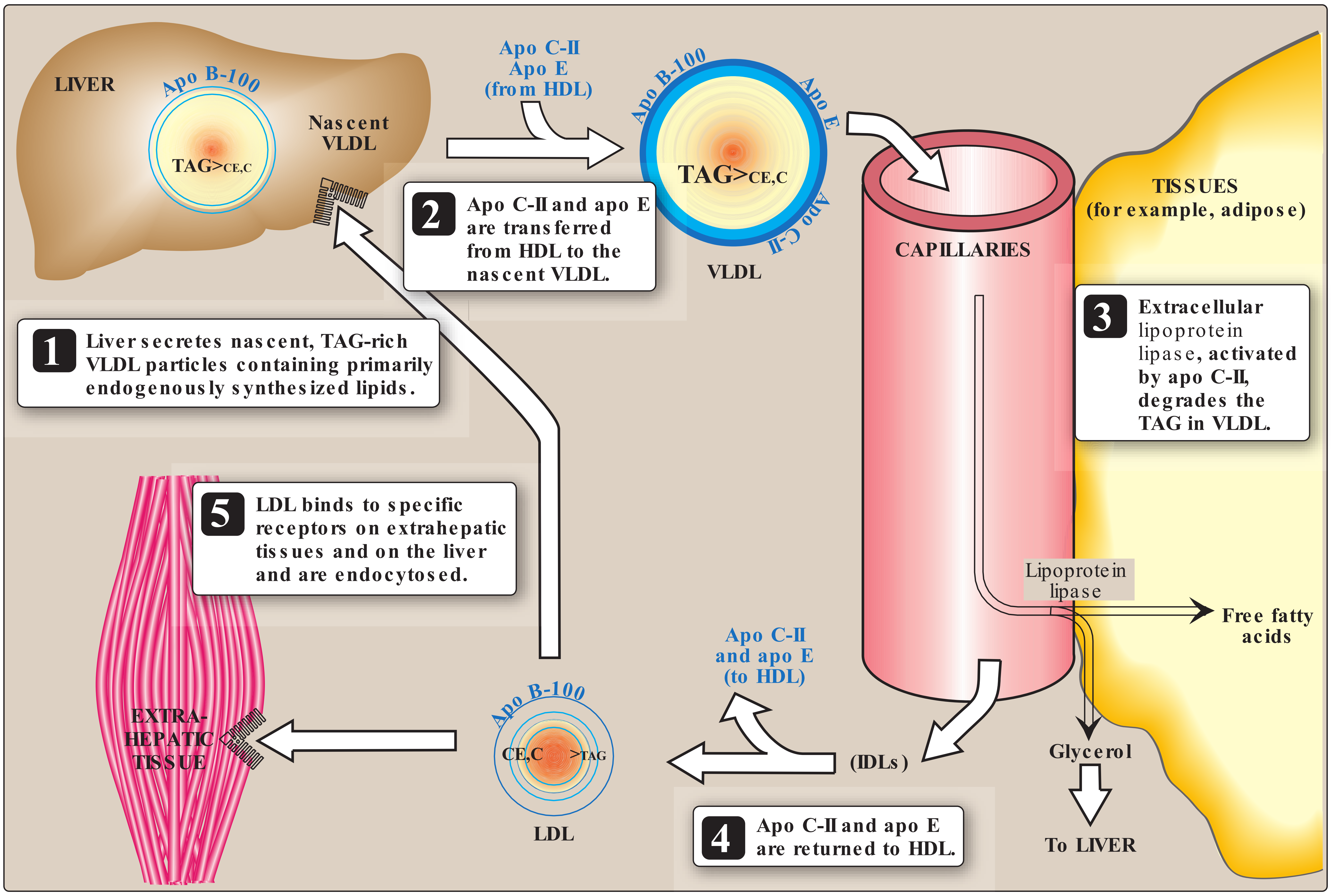
5. **Regulation of lipoprotein lipase activity:** LPL is synthesized by adipose tissue and by cardiac and skeletal muscle. Expression of the tissue-specific isozymes is regulated by nutritional state and hormonal level. For example, in the fed state (elevated insulin levels), LPL synthesis is increased in adipose but decreased in muscle tissue. Fasting (decreased insulin) favors LPL synthesis in muscle. [Note: The highest concentration of LPL is in cardiac muscle, reflecting the use of fatty acids to provide much of the energy needed for cardiac function.]

6. **Formation of chylomicron remnants:** As the chylomicron circulates, and more than 90% of the TAG in its core is degraded by LPL, the particle decreases in size and increases in density. In addition, the C apolipoproteins (but not apo E) are returned to HDL. The remaining particle, called a "remnant," is rapidly removed from the circulation by the liver, whose cell membranes

contain lipoprotein receptors that recognize apo E (see Figure 18.16). Chylomicron remnants bind to these receptors and are taken into the hepatocytes by endocytosis. The endocytosed vesicle then fuses with a lysosome, and the apolipoproteins, cholesteryl esters, and other components of the remnant are hydrolytically degraded, releasing amino acids, free cholesterol, and fatty acids. The receptor is recycled. (A more detailed discussion of the mechanism of receptor-mediated endocytosis is illustrated for LDL in Figure 18.20.)

C. Metabolism of very-low-density lipoproteins

VLDLs are produced in the liver (Figure 18.17). They are composed predominantly of endogenous TAG (approximately 60%), and their function is to carry this lipid from the liver (site of synthesis) to the peripheral tissues. There, the TAG is degraded by LPL, as discussed for chylomicrons (see p. 228). [Note: Nonalcoholic "fatty liver" (hepatic steatosis) occurs in conditions in which there is an


LIVER
Apo B-100
TAG>CE,C
Nascent VLDL
Apo C-II
Apo E
(from HDL)
Apo B-100
Apo E
TAG>CE,C
Apo C-II
VLDL
CAPILLARIES
TISSUES
(for example, adipose)
Lipoprotein lipase
Free fatty acids
Glycerol
To LIVER
Apo C-II
and apo E
(to HDL)
(IDLs)
Apo B-100
CE,C
>TAG
LDL
EXTRA-
HEPATIC
TISSUE

1 Liver secretes nascent, TAG-rich VLDL particles containing primarily endogenously synthesized lipids.

2 Apo C-II and apo E are transferred from HDL to the nascent VLDL.

3 Extracellular lipoprotein lipase, activated by apo C-II, degrades the TAG in VLDL.

4 Apo C-II and apo E are returned to HDL.

5 LDL binds to specific receptors on extrahepatic tissues and on the liver and are endocytosed.

Figure 18.17
Metabolism of very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL). Apo B-100, apo C-II, and apo E are apolipoproteins found as specific components of plasma lipoprotein particles. The lipoproteins are not drawn to scale (see Figure 18.13 for details of their size and density). TAG = triacylglycerol; HDL = high-density lipoprotein particle; IDLs = intermediate-density lipoprotein particles; C = cholesterol; CE = cholesteryl ester.

imbalance between hepatic TAG synthesis and the secretion of VLDL. Such conditions include obesity and type 2 diabetes mellitus.]

1. **Release from liver:** VLDLs are secreted directly into the blood by the liver as nascent particles containing apo B-100. They must obtain apo C-II and apo E from circulating HDL (see Figure 18.17). As with chylomicrons, apo C-II is required for activation of LPL. [Note: Abetalipoproteinemia is a rare hypolipoproteinemia caused by a defect in MTP, leading to an inability to load apo B with lipid. As a consequence, few VLDLs or chylomicrons are formed, and TAGs accumulate in the liver and intestine.]

2. **Modification in the circulation:** As VLDLs pass through the circulation, TAG is degraded by LPL, causing the VLDLs to decrease in size and become denser. Surface components, including the C and E apolipoproteins, are returned to HDL, but the particles retain apo B-100. Additionally, some TAGs are transferred from VLDL to HDL in an exchange reaction that concomitantly transfers cholesteryl esters from HDL to VLDL. This exchange is accomplished by cholesteryl ester transfer protein (CETP) as shown in Figure 18.18.

3. **Conversion to low-density lipoproteins:** With these modifications, the VLDL is converted in the plasma to LDL. Intermediate-sized particles, the intermediate-density lipoproteins (IDLs), or VLDL remnants, are observed during this transition. IDLs can also be taken up by liver cells through receptor-mediated endocytosis that uses apo E as the ligand. [Note: Apo E is normally present in three isoforms, E-2, (the least common), E-3 (the most common), and E-4. Apo E-2 binds poorly to receptors, and patients who are homozygotic for apo E-2 are deficient in the clearance of chylomicron remnants and IDLs. These individuals have familial type III hyperlipoproteinemia (familial dysbetalipoproteinemia, or broad beta disease), with hypercholesterolemia and premature atherosclerosis. Not as yet well understood is the fact that the E-4 isoform confers increased susceptibility to and decreased age of onset of the late-onset form of Alzheimer disease. The effect is dose dependent, with homozygotes being at greatest risk. Estimates of the risk vary.]

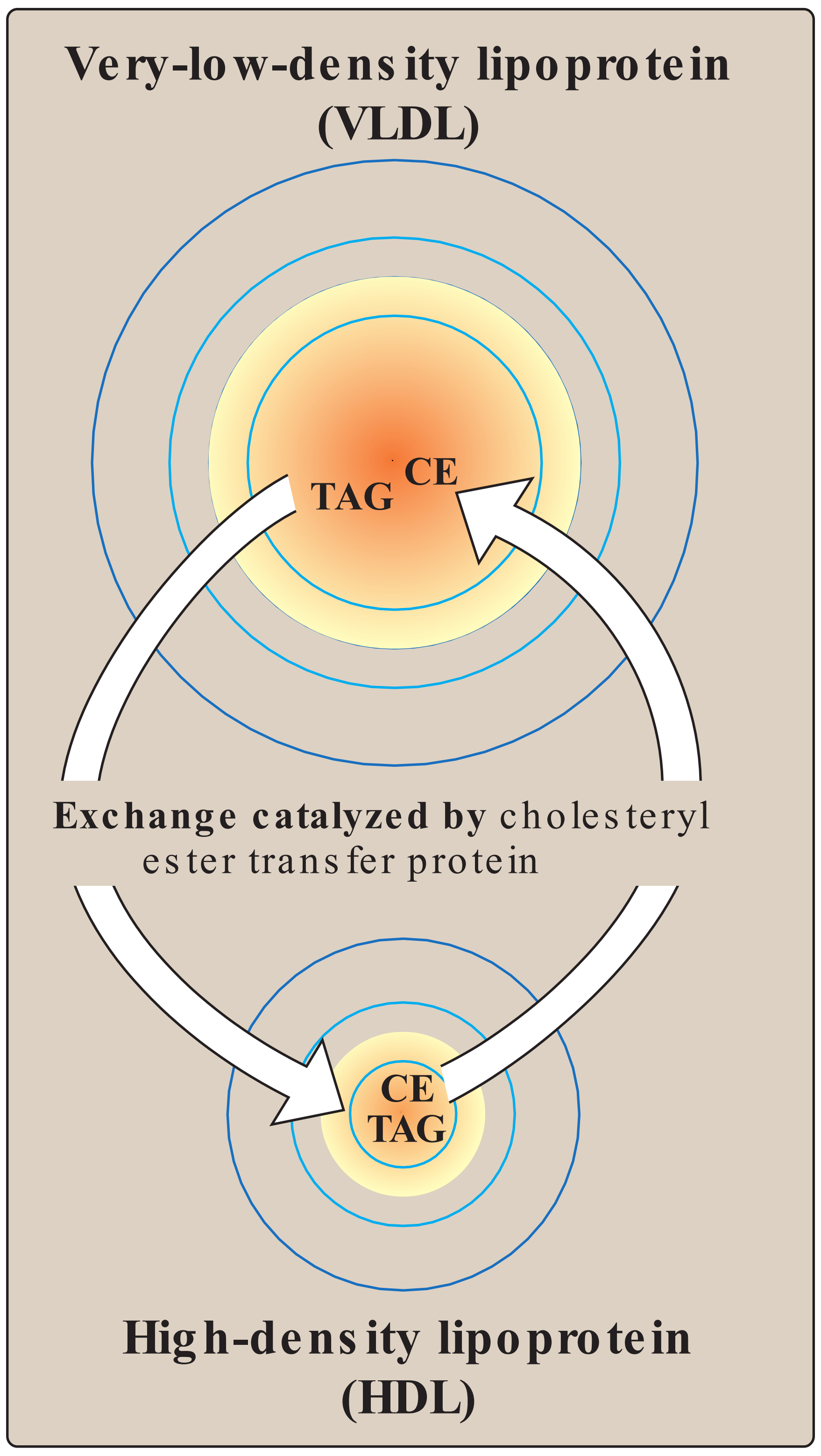

Very-low-density lipoprotein (VLDL)
TAG
CE
Exchange catalyzed by cholesteryl ester transfer protein
CE
TAG
High-density lipoprotein (HDL)

Figure 18.18
Transfer of cholesteryl ester (CE) from HDL to VLDL in exchange for triacylglycerol (TAG).

D. Metabolism of low-density lipoproteins

LDL particles contain much less TAG than their VLDL predecessors and have a high concentration of cholesterol and cholesteryl esters (Figure 18.19).

1. **Receptor-mediated endocytosis:** The primary function of LDL particles is to provide cholesterol to the peripheral tissues (or return it to the liver). They do so by binding to cell surface membrane LDL receptors that recognize apo B-100 (but not apo B-48). Because these LDL receptors can also bind apo E, they are known as apo B-100/apo E receptors. A summary of the uptake and degradation of LDL particles is presented in Figure 18.20. [Note: The numbers in brackets below refer to corresponding numbers on that figure.] A similar mechanism of receptor-mediated endocytosis is used for the cellular uptake and degradation of chylomicron remnants and IDLs by the liver.

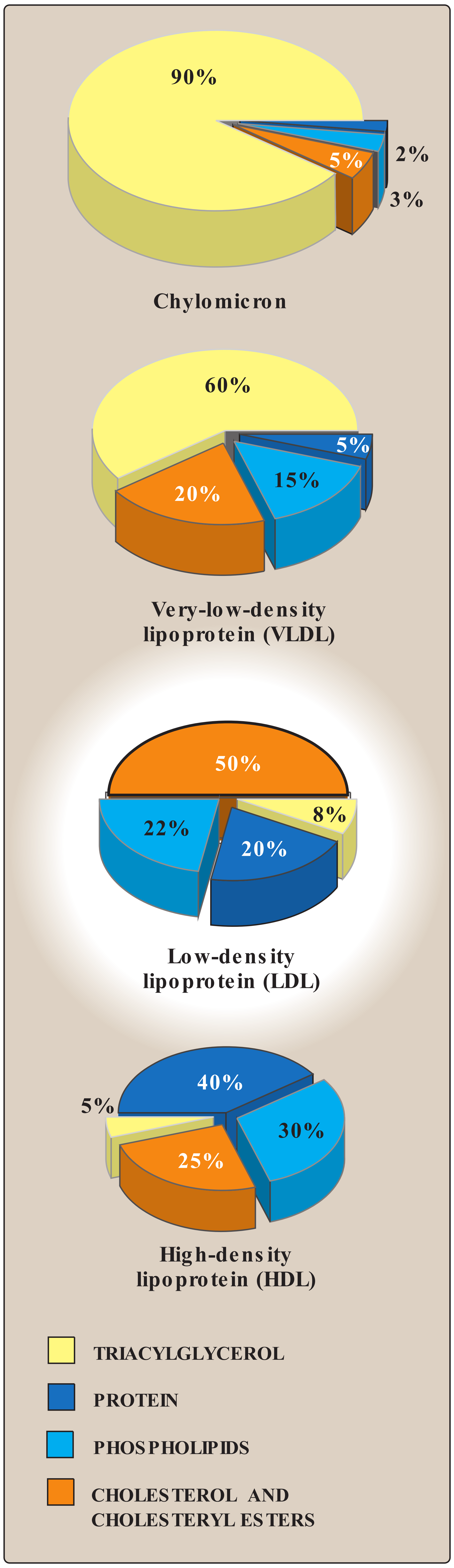


Figure 18.19
Composition of the plasma lipoproteins. Note the high concentration of cholesterol and cholesteryl esters in LDL.

[1] LDL receptors are negatively charged glycoproteins that are clustered in pits on cell membranes. The cytosolic side of the pit is coated with the protein clathrin, which stabilizes the pit.

[2] After binding, the LDL–receptor complex is taken in by endocytosis. [Note: A deficiency of functional LDL receptors causes a significant elevation in plasma LDL-C. Patients with such deficiencies have type II hyperlipidemia (familial hypercholesterolemia, or FH) and premature atherosclerosis. Autosomal-dominant hypercholesterolemia can also be caused by increased activity of a protease, proprotein convertase subtilisin/kexin type 9 (PCSK9), which promotes degradation of the receptor, and by defects in apo B-100 that reduce its binding to the receptor.]

[3] The vesicle containing LDL loses its clathrin coat and fuses with other similar vesicles, forming larger vesicles called endosomes.

[4] The pH of the endosome falls (due to the proton-pumping activity of endosomal ATPase), which allows separation of the LDL from its receptor. The receptors then migrate to one side of the endosome, whereas the LDLs stay free within the lumen of the vesicle. [Note: This structure is called CURL, the compartment for uncoupling of receptor and ligand.]

[5] The receptors can be recycled, whereas the lipoprotein remnants in the vesicle are transferred to lysosomes and degraded by lysosomal acid hydrolases, releasing free cholesterol, amino acids, fatty acids, and phospholipids. These compounds can be reutilized by the cell. [Note: Storage diseases caused by rare autosomal-recessive deficiencies in the ability to hydrolyze lysosomal cholesteryl esters (late-onset Wolman disease), or to transport free cholesterol out of the lysosome (Niemann-Pick disease, Type C) have been identified.]

2. **Effect of endocytosed cholesterol on cellular cholesterol homeostasis:** The chylomicron remnant-, IDL-, and LDL-derived cholesterol affects cellular cholesterol content in several ways (see Figure 18.20). First, expression of the gene for HMG CoA reductase is inhibited by high cholesterol, as a result of which, de novo cholesterol synthesis decreases. Additionally, degradation of the reductase is accelerated. Second, synthesis of new LDL receptor protein is reduced by decreasing the expression of the LDL receptor gene, thus limiting further entry of LDL cholesterol into cells. [Note: Regulation of the LDL receptor gene involves a SRE and SREBP-2, as was seen in the regulation of the gene for HMG CoA reductase (see p. 222). This allows coordinate regulation of the expression of these proteins.] Third, if the cholesterol is not required immediately for some structural or synthetic purpose, it is esterified by acyl CoA:cholesterol acyltransferase (ACAT). ACAT transfers a fatty acid from a fatty acyl CoA to cholesterol, producing a cholesteryl ester that can be stored in the cell (Figure 18.21). The activity of ACAT is enhanced in the presence of increased intracellular cholesterol.

3. **Uptake of chemically modified LDL by macrophage scavenger receptors:** In addition to the highly specific and regulated receptor-mediated pathway for LDL uptake described above,

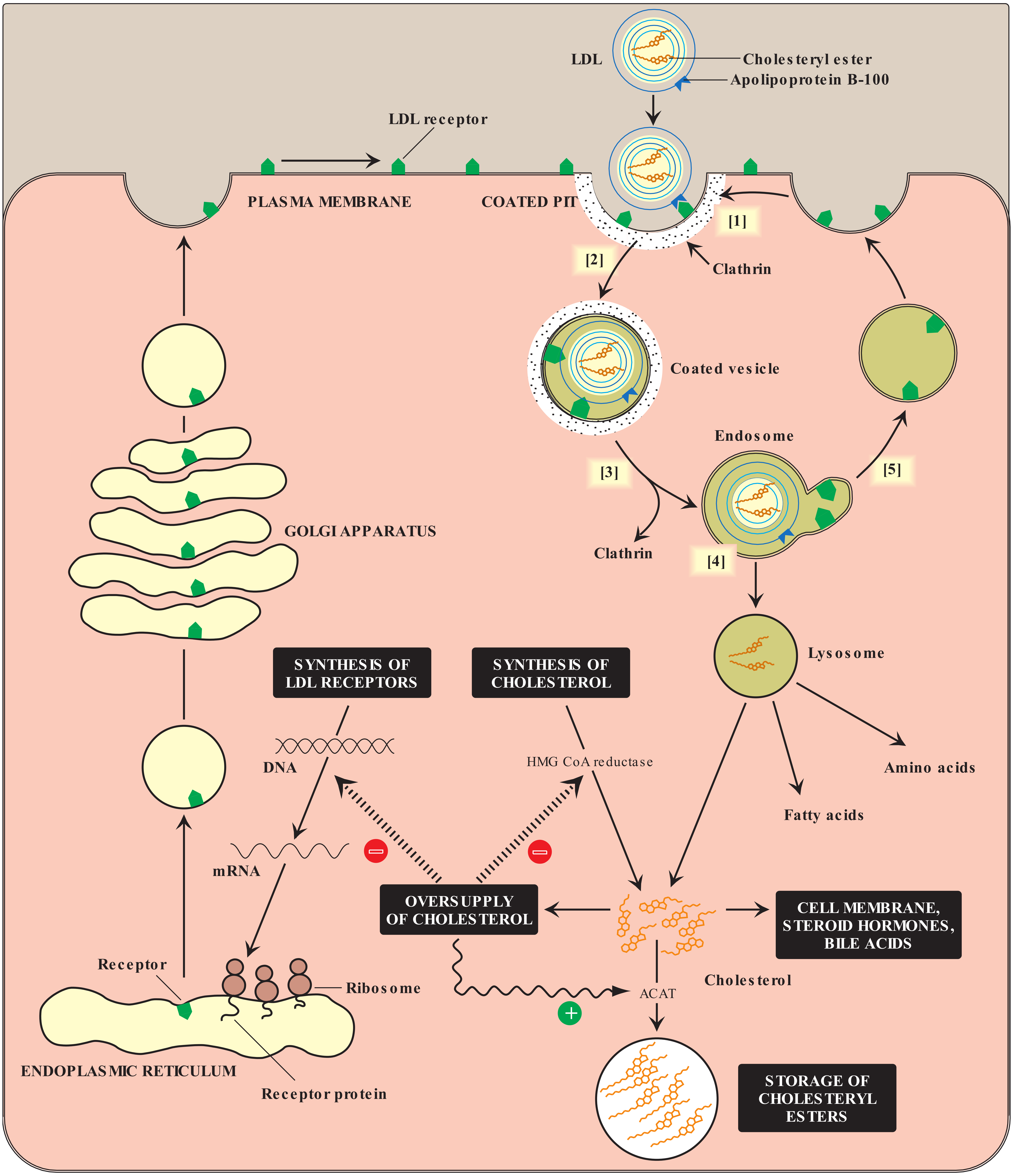

LDL
Cholesteryl ester
Apolipoprotein B-100
LDL receptor
PLASMA MEMBRANE
COATED PIT
[1]
[2]
Clathrin
Coated vesicle
Endosome
[3]
[5]
Clathrin
[4]
GOLGI APPARATUS
Lysosome
SYNTHESIS OF LDL RECEPTORS
SYNTHESIS OF CHOLESTEROL
DNA
HMG CoA reductase
Amino acids
Fatty acids
mRNA
OVERSUPPLY OF CHOLESTEROL
CELL MEMBRANE, STEROID HORMONES, BILE ACIDS
Receptor
Ribosome
ACAT
Cholesterol
ENDOPLASMIC RETICULUM
Receptor protein
STORAGE OF CHOLESTERYL ESTERS

Figure 18.20
Cellular uptake and degradation of low-density lipoprotein (LDL). [Note: Oversupply of cholesterol accelerates the degradation of HMG CoA reductase. It also decreases synthesis of the reductase by preventing expression of its gene as seen with the LDL receptor.] ACAT = acyl CoA:cholesterol acyltransferase; HMG CoA = hydroxymethylglutaryl coenzyme A; mRNA = messenger RNA.

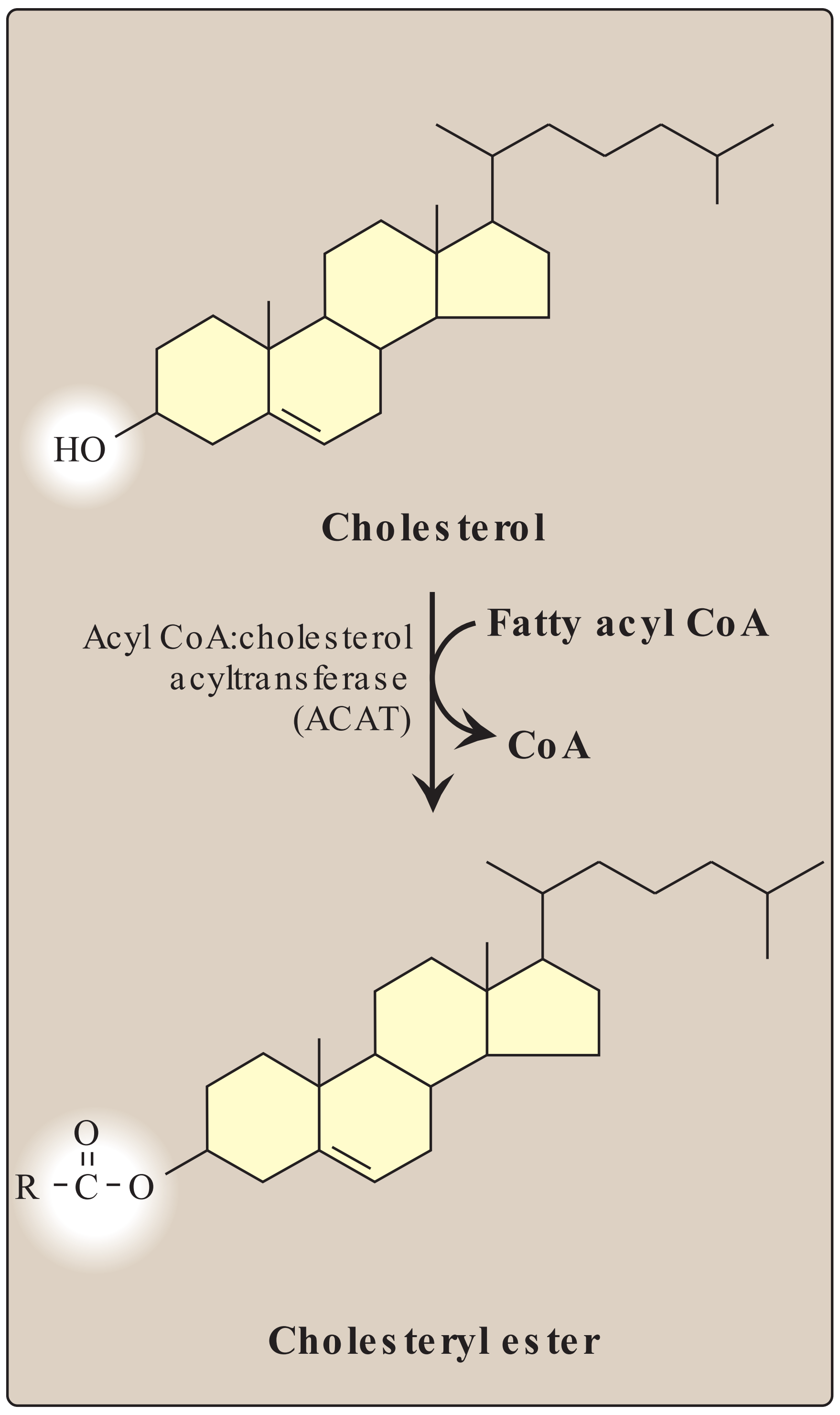


Figure 18.21
Synthesis of intracellular cholesteryl ester by ACAT. [Note: Lecithin:cholesterol acyl transferase (LCAT) is the extracellular enzyme that esterifies cholesterol using phosphatidylcholine (lecithin) as the source of the fatty acid.] CoA = coenzyme A.

macrophages possess high levels of scavenger receptor activity. These receptors, known as scavenger receptor class A (SR-A), can bind a broad range of ligands and mediate the endocytosis of chemically modified LDL in which the lipid components or apo B have been oxidized. Unlike the LDL receptor, the scavenger receptor is not downregulated in response to increased intracellular cholesterol. Cholesteryl esters accumulate in macrophages and cause their transformation into "foam" cells, which participate in the formation of atherosclerotic plaque (Figure 18.22).

E. Metabolism of high-density lipoproteins

HDLs comprise a heterogeneous family of lipoproteins with a complex metabolism that is not yet completely understood. HDL particles are formed in blood by the addition of lipid to apo A-1, an apolipoprotein made by the liver and intestine and secreted into blood. [Note: HDLs are also formed within the liver and intestine.] Apo A-1 accounts for about 70% of the apolipoproteins in HDL. HDLs perform a number of important functions, including the following.

1. **Apolipoprotein supply:** HDL particles serve as a circulating reservoir of apo C-II (the apolipoprotein that is transferred to VLDL and chylomicrons and is an activator of LPL) and apo E (the apolipoprotein required for the receptor-mediated endocytosis of IDLs and chylomicron remnants).

2. **Uptake of unesterified cholesterol:** Nascent HDLs are disc-shaped particles containing primarily phospholipid (largely phosphatidylcholine) and apolipoproteins A, C, and E. They take up cholesterol from nonhepatic (peripheral) tissues and return it to the liver as cholesteryl esters (Figure 18.23). [Note: HDL particles are excellent acceptors of unesterified cholesterol as a result of their high concentration of phospholipids, which are important solubilizers of cholesterol.]

3. **Esterification of cholesterol:** When cholesterol is taken up by HDL, it is immediately esterified by the plasma enzyme lecithin:cholesterol acyltransferase (LCAT, also known as PCAT, in which "P" stands for phosphatidylcholine, the source of the fatty acid). This enzyme is synthesized and secreted by the liver. LCAT binds to nascent HDL, and is activated by apo A-I. LCAT transfers the fatty acid from carbon 2 of phosphatidylcholine to cholesterol. This produces a hydrophobic cholesteryl ester, which is sequestered in the core of the HDL, and lysophosphatidylcholine, which binds to albumin. [Note: Esterification maintains the cholesterol concentration gradient, allowing continued efflux of cholesterol to HDL.] As the discoidal nascent HDL accumulates cholesteryl esters, it first becomes a spherical, relatively cholesteryl ester–poor HDL3 and, eventually, a cholesteryl ester–rich HDL2 particle that carries these esters to the liver. CETP (see p. 231) moves some of the cholesteryl esters from HDL to VLDL in exchange for TAG, relieving product inhibition of LCAT. Because VLDLs are catabolized to LDL, the cholesteryl esters transferred by CETP are ultimately taken up by the liver (see p. 236).

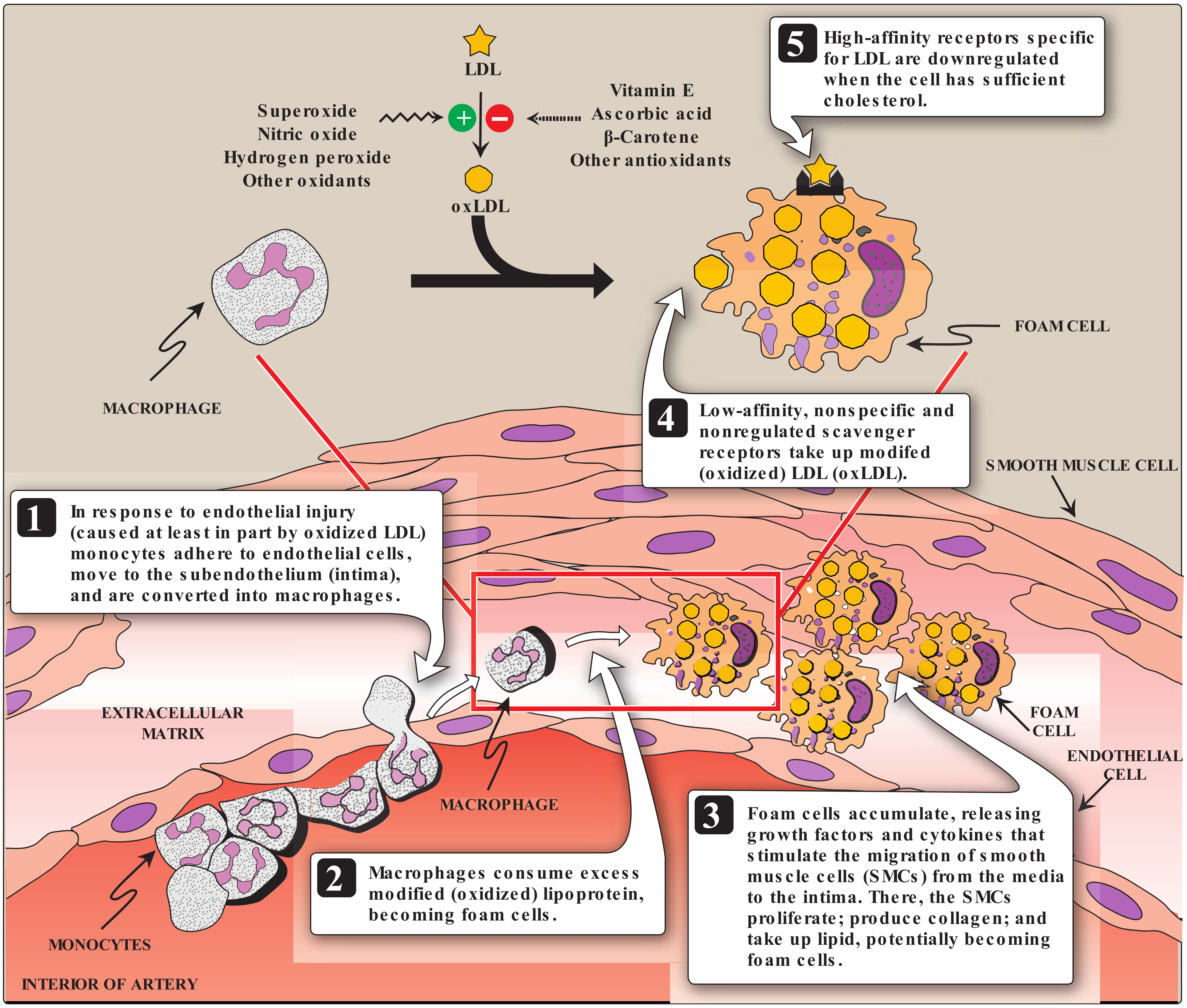

LDL
Superoxide
Nitric oxide
Hydrogen peroxide
Other oxidants
Vitamin E
Ascorbic acid
β-Carotene
Other antioxidants
oxLDL
5 High-affinity receptors specific for LDL are downregulated when the cell has sufficient cholesterol.
FOAM CELL
MACROPHAGE
4 Low-affinity, nonspecific and nonregulated scavenger receptors take up modifed (oxidized) LDL (oxLDL).
SMOOTH MUSCLE CELL
1 In response to endothelial injury (caused at least in part by oxidized LDL) monocytes adhere to endothelial cells, move to the subendothelium (intima), and are converted into macrophages.
EXTRACELLULAR MATRIX
MACROPHAGE
FOAM CELL
ENDOTHELIAL CELL
2 Macrophages consume excess modified (oxidized) lipoprotein, becoming foam cells.
3 Foam cells accumulate, releasing growth factors and cytokines that stimulate the migration of smooth muscle cells (SMCs) from the media to the intima. There, the SMCs proliferate; produce collagen; and take up lipid, potentially becoming foam cells.
MONOCYTES
INTERIOR OF ARTERY

Figure 18.22
Role of oxidized lipoproteins in plaque formation in an arterial wall. LDL = low-density lipoprotein.

4. **Reverse cholesterol transport:** The selective transfer of cholesterol from peripheral cells to HDL, from HDL to the liver for bile acid synthesis or disposal via the bile, and to steroidogenic cells for hormone synthesis, is a key component of cholesterol homeostasis. This process of reverse cholesterol transport is, in part, the basis for the inverse relationship seen between plasma HDL concentration and atherosclerosis and for HDL's designation as the "good" cholesterol carrier. [Note: Exercise and estrogen raise HDL levels.] Reverse cholesterol transport involves efflux of cholesterol from peripheral cells to HDL, esterification of the cholesterol by LCAT, binding of the cholesteryl ester–rich HDL (HDL2) to liver (and steroidogenic cells), the selective transfer of the cholesteryl esters into these cells, and the release of

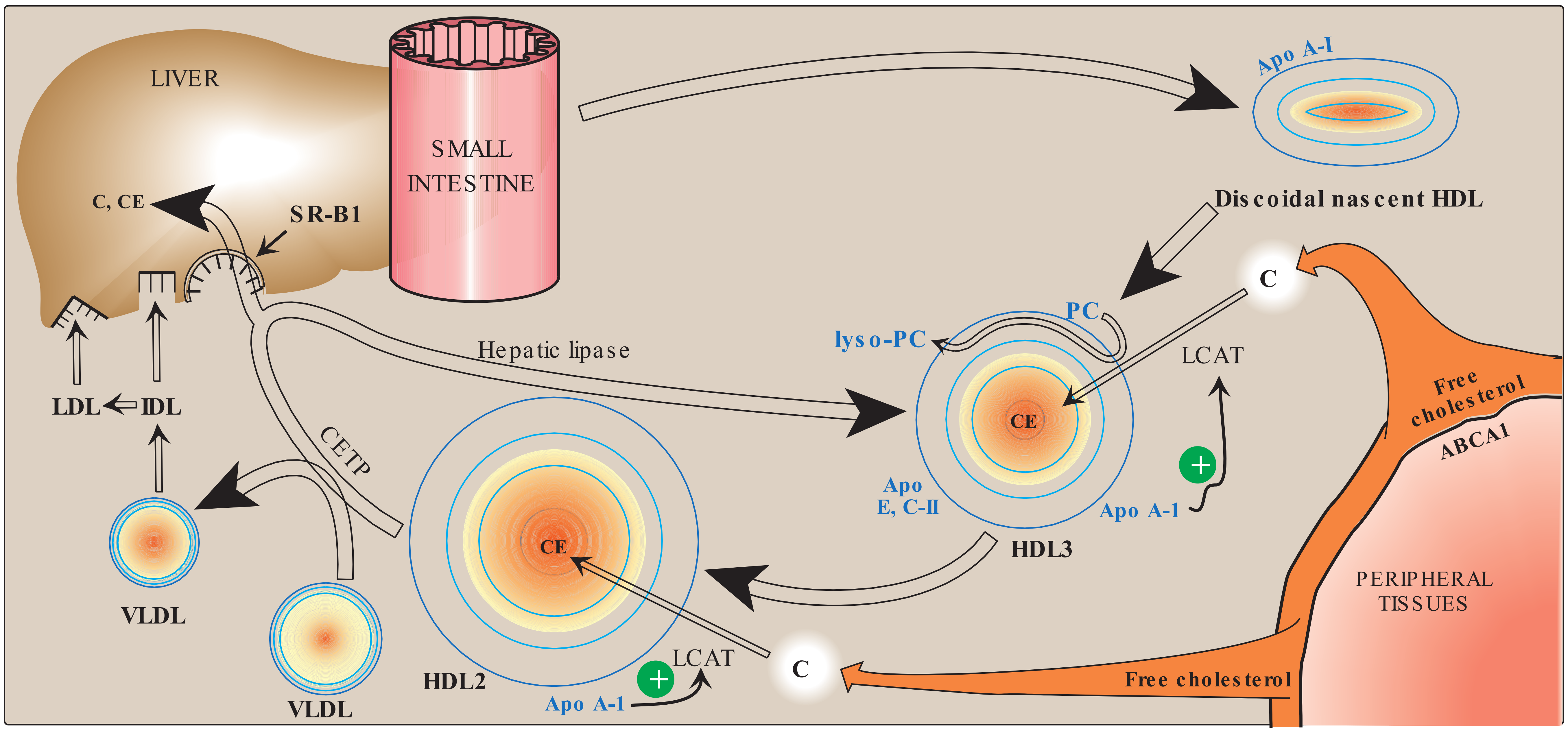

LIVER
SMALL INTESTINE
Apo A-I
Discoidal nascent HDL
C, CE
SR-B1
C
PC
lyso-PC
LCAT
Hepatic lipase
CE
LDL
IDL
CETP
Free cholesterol
ABCA1
Apo E, C-II
Apo A-1
HDL3
VLDL
CE
PERIPHERAL TISSUES
LCAT
C
HDL2
Free cholesterol
VLDL
Apo A-1

Figure 18.23
Metabolism of high-density lipoprotein (HDL) particles. Apo = apolipoprotein; ABCA1 = transport protein; C = cholesterol; CE = cholesteryl ester; LCAT = lecithin:cholesterol acyltransferase; VLDL = very-low-density lipoprotein; IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein; CETP = cholesteryl ester transfer protein; SR-B1 = scavenger receptor B1.

lipid-depleted HDL (HDL3). The efflux of cholesterol from peripheral cells is mediated, at least in part, by the transport protein ABCA1. [Note: Tangier disease is a very rare deficiency of ABCA1 and is characterized by the virtual absence of HDL particles due to degradation of lipid-poor apo A-1.] The uptake of cholesteryl esters by the liver is mediated by a cell-surface receptor, SR-B1 (scavenger receptor class B type 1) that binds HDL (see p. 234 for SR-A receptors). The HDL particle itself is not taken up. Instead, there is selective uptake of the cholesteryl ester from the HDL particle. [Note: Hepatic lipase, with its ability to degrade both TAG and phospholipids, also participates in the conversion of HDL2 to HDL3.]

ABCA1 is an ATP-binding cassette (ABC) protein. ABC proteins use energy from ATP hydrolysis to transport materials, including lipids, in and out of cells and across intracellular compartments. In addition to Tangier disease, defects in specific ABC proteins result in sitosterolemia, X-linked adrenoleukodystrophy, respiratory distress syndrome due to decreased surfactant secretion, and cystic fibrosis.

F. Role of lipoprotein (a) in heart disease

Lipoprotein (a), or Lp(a), is a particle that, when present in large quantities in the plasma, is associated with an increased risk of coronary heart disease. Lp(a) is nearly identical in structure to an LDL particle.

Its distinguishing feature is the presence of an additional apolipoprotein molecule, apo(a), that is covalently linked at a single site to apo B-100. Circulating levels of Lp(a) are determined primarily by genetics. However, factors such as diet may play some role, as trans fatty acids have been shown to increase Lp(a), whereas ω-3 fatty acids decrease it. [Note: Apo(a) is structurally homologous to plasminogen, the precursor of a blood protease whose target is fibrin, the main protein component of blood clots (See Chapter 34 online). It is hypothesized that elevated Lp(a) slows the breakdown of blood clots that trigger heart attacks because it competes with plasminogen for binding to fibrin. The physiologic function of Lp(a) in unknown. Niacin reduces Lp(a), as well as LDL-cholesterol and TAGs, and raises HDL.]

VII. STEROID HORMONES

Cholesterol is the precursor of all classes of steroid hormones: glucocorticoids (for example, cortisol), mineralocorticoids (for example, aldosterone), and the sex hormones (that is, androgens, estrogens, and progestins) as shown in Figure 18.24). [Note: Glucocorticoids and mineralocorticoids are collectively called corticosteroids.] Synthesis and secretion occur in the adrenal cortex (cortisol, aldosterone, and androgens), ovaries and placenta (estrogens and progestins), and testes (testosterone). Steroid hormones are transported by the blood from their sites of synthesis to their target organs. Because of their hydrophobicity, they must be complexed with a plasma protein. Plasma albumin can act as a nonspecific carrier and does carry aldosterone. However, specific steroid-carrier plasma proteins bind the steroid hormones more tightly than does albumin (for example, corticosteroid-binding globulin, or transcortin, is responsible for transporting cortisol). A number of genetic diseases are caused by deficiencies in specific steps in the biosynthesis of steroid hormones. Some representative diseases are described in Figure 18.25.

A. Synthesis of steroid hormones

Synthesis involves shortening the hydrocarbon chain of cholesterol and hydroxylation of the steroid nucleus. The initial and rate-limiting reaction converts cholesterol to the 21-carbon pregnenolone. It is catalyzed by the cholesterol side-chain cleavage enzyme (desmolase, $P450_{scc}$), a cytochrome P450 (CYP) mixed-function oxidase of the inner mitochondrial membrane (see p. 149). NADPH and O_2 are required for the reaction. The cholesterol substrate can be newly synthesized, taken up from lipoproteins, or released by an esterase from cholesteryl esters stored in the cytosol of steroidogenic tissues. The cholesterol moves to the outer mitochondrial membrane. An important control point is the subsequent movement from the outer to the inner mitochondrial membrane. This process is mediated by StAR (steroidogenic acute regulatory) protein.

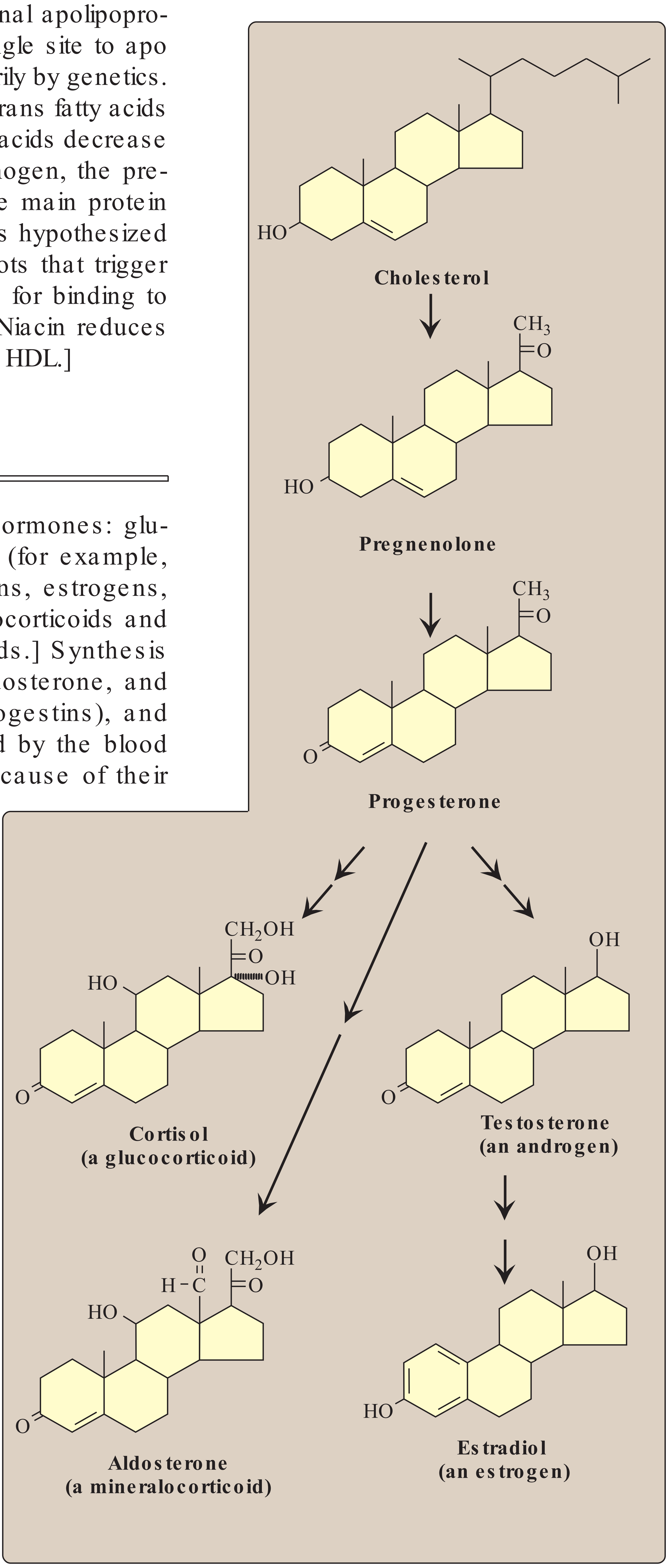
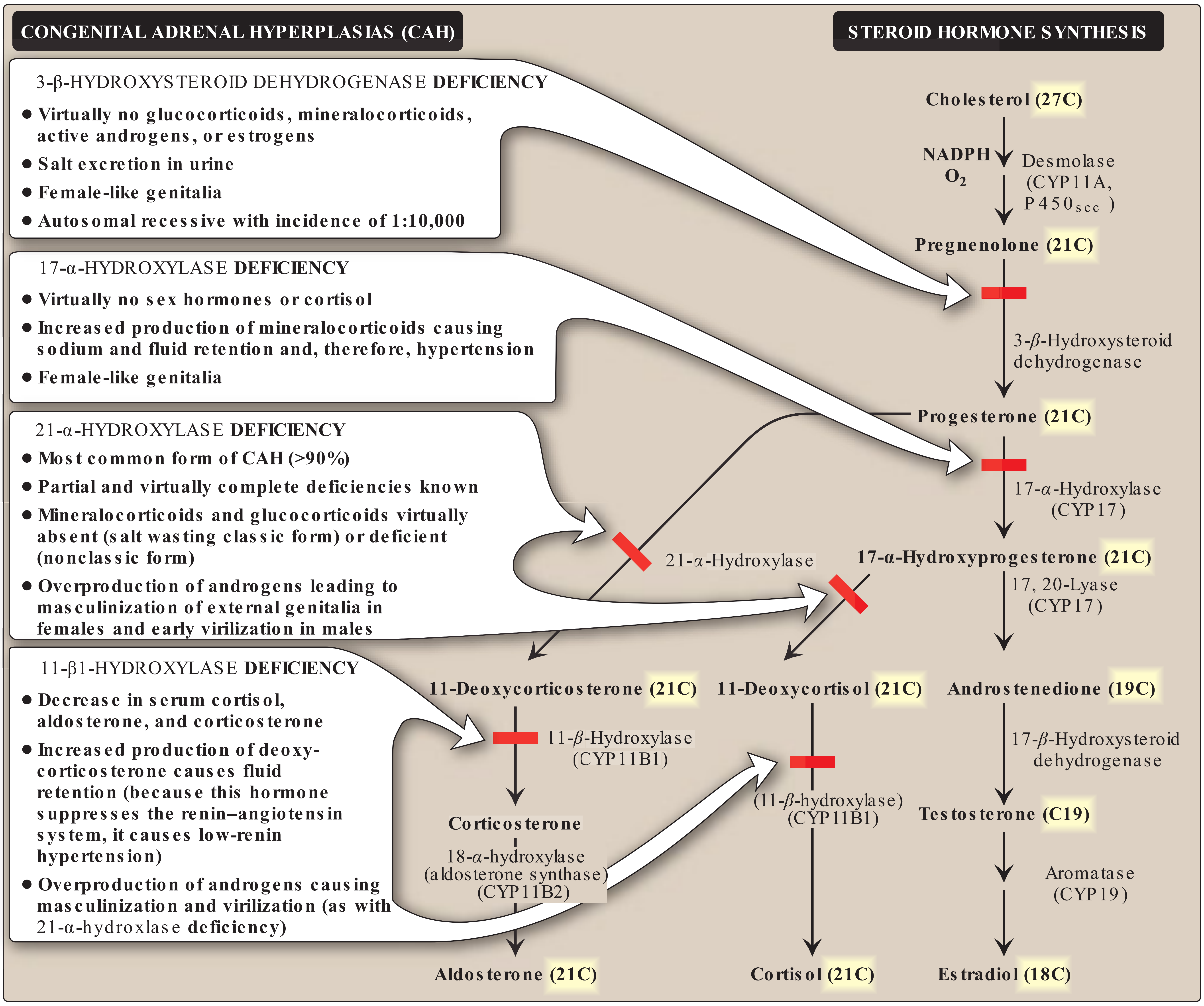


Figure 18.24
Key steroid hormones.


CONGENITAL ADRENAL HYPERPLASIAS (CAH)
STEROID HORMONE SYNTHESIS
Cholesterol (27C)
NADPH
O_2
Desmolase (CYP11A, $P450_{scc}$)
Pregnenolone (21C)
3-β-Hydroxysteroid dehydrogenase
Progesterone (21C)
17-α-Hydroxylase (CYP17)
21-α-Hydroxylase
17-α-Hydroxyprogesterone (21C)
17, 20-Lyase (CYP17)
11-Deoxycorticosterone (21C)
11-Deoxycortisol (21C)
Androstenedione (19C)
11-β-Hydroxylase (CYP11B1)
(11-β-hydroxylase) (CYP11B1)
17-β-Hydroxysteroid dehydrogenase
Corticosterone
Testosterone (C19)
18-α-hydroxylase (aldosterone synthase) (CYP11B2)
Aromatase (CYP19)
Aldosterone (21C)
Cortisol (21C)
Estradiol (18C)

3-β-HYDROXYSTEROID DEHYDROGENASE **DEFICIENCY**

- **Virtually no glucocorticoids, mineralocorticoids, active androgens, or estrogens**
- **Salt excretion in urine**
- **Female-like genitalia**
- **Autosomal recessive with incidence of 1:10,000**

17-α-HYDROXYLASE **DEFICIENCY**

- **Virtually no sex hormones or cortisol**
- **Increased production of mineralocorticoids causing sodium and fluid retention and, therefore, hypertension**
- **Female-like genitalia**

21-α-HYDROXYLASE **DEFICIENCY**

- **Most common form of CAH (>90%)**
- **Partial and virtually complete deficiencies known**
- **Mineralocorticoids and glucocorticoids virtually absent (salt wasting classic form) or deficient (nonclassic form)**
- **Overproduction of androgens leading to masculinization of external genitalia in females and early virilization in males**

11-β1-HYDROXYLASE **DEFICIENCY**

- **Decrease in serum cortisol, aldosterone, and corticosterone**
- **Increased production of deoxycorticosterone causes fluid retention (because this hormone suppresses the renin–angiotensin system, it causes low-renin hypertension)**
- **Overproduction of androgens causing masculinization and virilization (as with** 21-α-hydroxlase **deficiency)**

Figure 18.25
Steroid hormone synthesis and associated diseases. [Note: 3-*β*-Hydroxysteroid dehydrogenase, CYP17, and CYP11B2 are bifunctional enzymes. Synthesis of testosterone and the estrogens from cholesterol occurs primarily outside of the adrenal gland.] NADPH = nicotinamide adenine dinucleotide phosphate; CYP = cytochrome P450.

Pregnenolone is the parent compound for all steroid hormones (see Figure 18.25). It is oxidized and then isomerized to progesterone, which is further modified to the other steroid hormones by hydroxylation reactions that occur in the ER and mitochondria. Like desmolase, the enzymes primarily are CYP proteins. A defect in the activity or amount of an enzyme in this pathway can lead to a deficiency in the synthesis of hormones beyond the affected step and to an excess in the hormones or metabolites before that step. Because all members of the pathway have potent biologic activity, serious metabolic imbalances occur with enzyme deficiencies (see Figure 18.25). Collectively these disorders are known as the congenital adrenal hyperplasias. [Note: Addison disease, due to autoimmune destruction of the adrenal cortex, is characterized by adrenocortical insufficiency.]

B. Secretion of adrenal cortical steroid hormones

Steroid hormones are secreted on demand from their tissues of origin in response to hormonal signals. The corticosteroids and androgens are made in different regions of the adrenal cortex and are secreted into blood in response to different signals.

1. **Cortisol:** Its production in the middle layer (zona fasciculata) of the adrenal cortex is controlled by the hypothalamus, to which the pituitary gland is attached (Figure 18.26). In response to severe stress (for example, infection), corticotropin-releasing hormone (CRH), produced by the hypothalamus, travels through capillaries to the anterior lobe of the pituitary, where it induces the production and secretion of adrenocorticotropic hormone (ACTH). The polypeptide ACTH, the “stress hormone,” stimulates the adrenal cortex to synthesize and secrete the glucocorticoid cortisol. Cortisol allows the body to respond to stress through its effects on intermediary metabolism (for example, increased gluconeogenesis) and the inflammatory and immune responses. As cortisol levels rise, the release of CRH and ACTH is inhibited. [Note: ACTH binds to a membrane G protein–coupled receptor, resulting in cyclic AMP (cAMP) production and activation of protein kinase A (PKA) (see p. 94). PKA phosphorylates and activates both the esterase that converts cholesteryl ester to free cholesterol and StAR protein.]
2. **Aldosterone:** Its production in the outer layer (zona glomerulosa) of the adrenal cortex is induced by a decrease in the plasma Na^+/K^+ ratio, and by the hormone angiotensin II. Angiotensin II (an octapeptide) is produced from angiotensin I (a decapeptide) by angiotensin-converting enzyme (ACE), an enzyme found predominantly in the lungs but also distributed widely in the body. [Note: Angiotensin I is produced in the blood by cleavage of an inactive precursor, angiotensinogen, secreted by the liver. Cleavage is accomplished by the enzyme renin, made and secreted by the kidney.] Angiotensin II binds to cell-surface receptors. However, in contrast to ACTH, its effects are mediated through the phosphatidylinositol 4,5-bisphosphate pathway (see p. 205) and not by cAMP. Aldosterone’s primary effect is on the kidney tubules, where it stimulates sodium and water uptake and potassium excretion (Figure 18.27). [Note: An effect of aldosterone is an increase in blood pressure. Competitive inhibitors of ACE are used to treat renin-dependent hypertension.]
3. **Androgens:** Both the inner (zona reticularis) and middle layers of the adrenal cortex produce androgens, primarily dehydroepiandrosterone and androstenedione. Although adrenal androgens themselves are weak, they are converted in peripheral tissues to testosterone, a stronger androgen, and to estrogens.

> Estrogens are derived from androstenedione and testosterone by aromatase (CYP19). Aromatase inhibitors are used in the treatment of estrogen-responsive breast cancer in postmenopausal women.

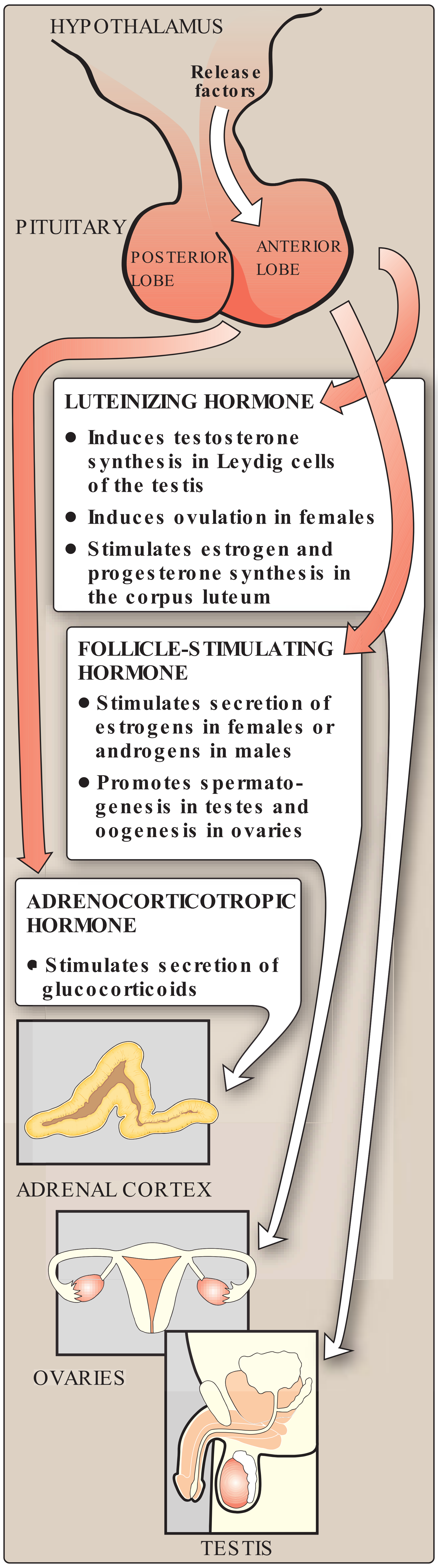
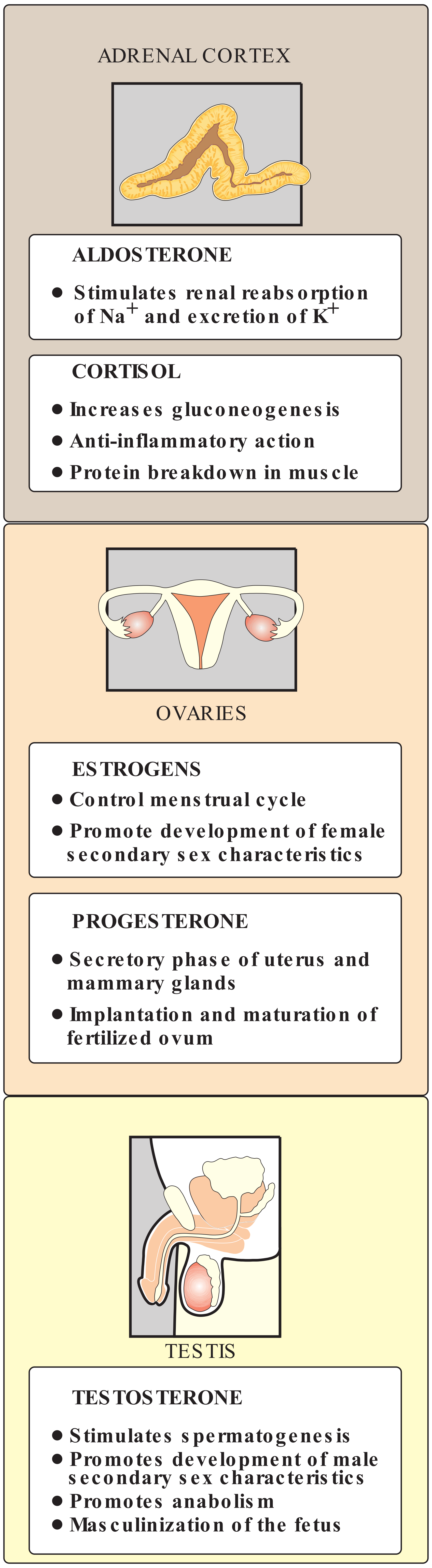

HYPOTHALAMUS
Release factors
PITUITARY
POSTERIOR LOBE
ANTERIOR LOBE
LUTEINIZING HORMONE
• Induces testosterone synthesis in Leydig cells of the testis
• Induces ovulation in females
• Stimulates estrogen and progesterone synthesis in the corpus luteum
FOLLICLE-STIMULATING HORMONE
• Stimulates secretion of estrogens in females or androgens in males
• Promotes spermatogenesis in testes and oogenesis in ovaries
ADRENOCORTICOTROPIC HORMONE
• Stimulates secretion of glucocorticoids
ADRENAL CORTEX
OVARIES
TESTIS

Figure 18.26
Pituitary hormone stimulation of steroid hormone synthesis and secretion.



ALDOSTERONE

- Stimulates renal reabsorption of Na^+ and excretion of K^+

CORTISOL

- Increases gluconeogenesis
- Anti-inflammatory action
- Protein breakdown in muscle

ESTROGENS

- Control menstrual cycle
- Promote development of female secondary sex characteristics

PROGESTERONE

- Secretory phase of uterus and mammary glands
- Implantation and maturation of fertilized ovum

TESTOSTERONE

- Stimulates spermatogenesis
- Promotes development of male secondary sex characteristics
- Promotes anabolism
- Masculinization of the fetus

Figure 18.27
Actions of steroid hormones.

C. Secretion of steroid hormones from gonads

The testes and ovaries synthesize hormones necessary for sexual differentiation and reproduction. A single hypothalamic-releasing factor, gonadotropin-releasing hormone, stimulates the anterior pituitary to release the glycoproteins luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Like ACTH, LH and FSH bind to surface receptors and cause an increase in cAMP. LH stimulates the testes to produce testosterone and the ovaries to produce estrogens and progesterone (see Figure 18.27). FSH regulates the growth of ovarian follicles and stimulates testicular spermatogenesis.

D. Mechanism of steroid hormone action

Each steroid hormone diffuses across the plasma membrane of its target cell and binds to a specific cytosolic or nuclear receptor. These receptor–ligand complexes accumulate in the nucleus, dimerize, and bind to specific regulatory DNA sequences (hormone-response elements [HREs]) in association with coactivator proteins, thereby causing promoter activation and increased transcription of targeted genes (Figure 18.28). An HRE is found in the promoter or an enhancer element (see p. 424) for genes that respond to a specific steroid hormone, thus insuring coordinated regulation of these genes. Hormone–receptor complexes can also inhibit transcription in association with corepressors. [Note: The binding of a hormone to its receptor causes a conformational change in the receptor that uncovers its DNA-binding domain, allowing the complex to interact through a zinc-finger motif with the appropriate sequence on the DNA. Receptors for the steroid hormones, plus those for thyroid hormone, retinoic acid (see p. 382), and 1,25-dihydroxycholecalciferol (vitamin D, see p. 386), are members of a "superfamily" of structurally related gene regulators that function in a similar way.]

E. Further metabolism of steroid hormones

Steroid hormones are generally converted into inactive metabolic excretion products in the liver. Reactions include reduction of unsaturated bonds and the introduction of additional hydroxyl groups. The resulting structures are made more soluble by conjugation with glucuronic acid or sulfate (from 3'-phosphoadenosyl-5'-phosphosulfate, see p. 162). Approximately 20%–30% of these metabolites are secreted into the bile and then excreted in the feces, whereas the remainder are released into the blood and filtered from the plasma in the kidney, passing into the urine. These conjugated metabolites are fairly water soluble and do not need protein carriers.

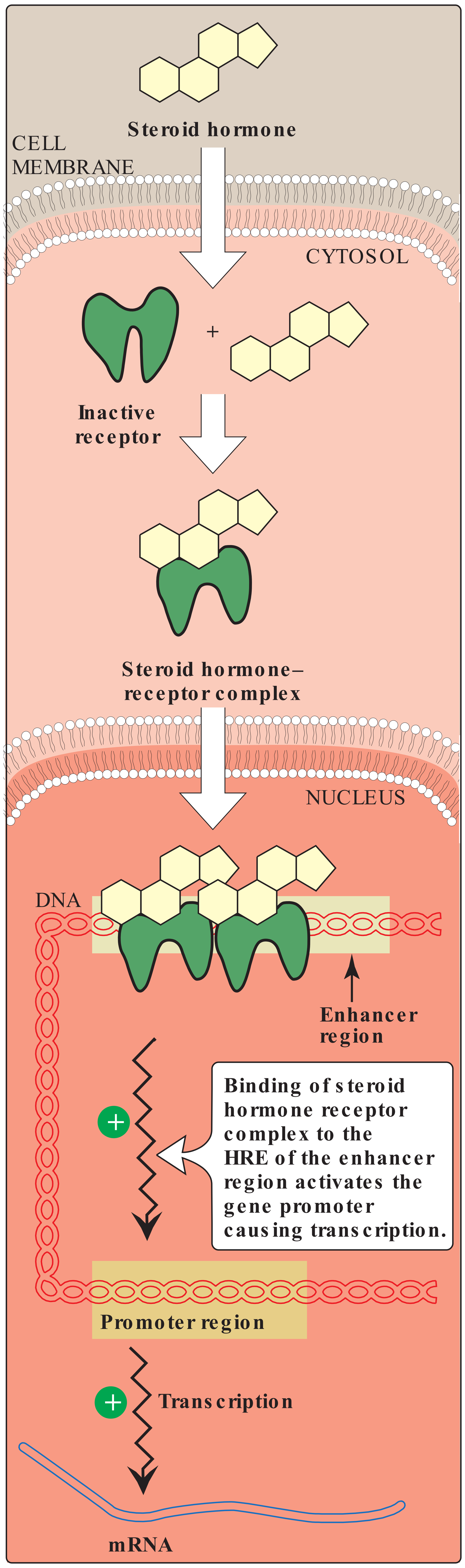
VIII. CHAPTER SUMMARY

Cholesterol is a **hydrophobic** compound, with a single hydroxyl group located at carbon 3 of the A ring, to which a fatty acid can be attached, producing an even more hydrophobic **cholesteryl ester**. Cholesterol is synthesized by virtually all human tissues, although primarily by **liver**, **intestine**, **adrenal cortex**, and **reproductive tissues** (Figure 18.29). All the carbon atoms in cholesterol are provided by **acetyl coenzyme A (CoA)**, and **nicotinamide adenine dinucleotide phosphate** provides the reducing equivalents. The pathway is driven by hydrolysis of the high-energy thioester bond of acetyl CoA and the terminal phosphate bond of adenosine triphosphate. Cholesterol is synthesized in the **cyto-**

plasm.The **rate-limiting and regulated step** in cholesterol synthesis is catalyzed by the smooth endoplasmic reticulum–membrane protein, hydroxymethylglutaryl coenzyme A (HMG CoA) reductase, which produces **mevalonate** from HMG CoA. The enzyme is regulated by a number of mechanisms: 1) **Expression of the gene for the** reductase is activated when cholesterol levels are low, via the transcription factor, **sterol regulatory element–binding protein-2** (**SREBP-2**), bound to a sterol regulatory element (**SRE**), resulting in increased enzyme and, therefore, more cholesterol, synthesis; 2) **degradation** of the reductase is accelerated when cholesterol levels are high; 3) reductase activity is controlled by adenosine monophosphate (AMP)–activated protein kinase ([AMPK], which phosphorylates and **inactivates** the reductase) and an **insulin-activated protein** phosphatase (which dephosphorylates and **activates** it); and 4) expression of the gene for the reductase is upregulated by insulin and downregulated by glucagon. **Statins** are **competitive inhibitors** of HMG CoA reductase. These drugs are used to decrease plasma cholesterol in patients with **hypercholesterolemia**. The ring structure of cholesterol cannot be degraded in humans.

Cholesterol can be eliminated from the body either by **conversion to bile salts** or by **secretion into the bile**. **Bile salts** and **phosphatidylcholine** are quantitatively the most important organic components of bile. The rate-limiting step in bile acid synthesis is catalyzed by cholesterol-7-α-hydroxylase, which is **inhibited** by **bile acids**. Before the bile acids leave the liver, they are conjugated to a molecule of either **glycine** or **taurine**, producing the **conjugated bile salts glycocholic** or **taurocholic acid** and **glycochenodeoxycholic** or **taurochenodeoxycholic acid**. Bile salts (deprotonated) are more amphipathic than bile acids (protonated) and, therefore, are more effective emulsifiers of dietary fat. In the intestine, bacteria can remove the glycine and taurine and can remove a hydroxyl group from the steroid nucleus, producing the **secondary bile salts**, **deoxycholic** and **lithocholic acids**. More than 95% of the bile salts are efficiently reabsorbed in the intestinal ileum by a sodium–bile salt cotransporter, returned to the blood, and carried by albumin back to the liver where they are taken up by the hepatic form of the cotransporter and reused (**enterohepatic circulation, which bile acid sequestrants reduce**). If more cholesterol enters the bile than can be solubilized by the available bile salts and phosphatidylcholine, **cholesterol gallstone disease** (**cholelithiasis**) can occur.

The plasma lipoproteins include **chylomicrons**, **very-low-density lipoproteins** (**VLDLs**), **low-density lipoproteins** (**LDLs**), and **high-density lipoproteins** (**HDLs**). They function to keep lipids (primarily **triacylglycerol** [TAG] and **cholesteryl esters**) soluble as they transport them between tissues. Lipoproteins are composed of a **neutral lipid** (TAG, **cholesteryl esters**, or both) core surrounded by a shell of amphipathic **apolipoproteins**, **phospholipid**, and **unesterified cholesterol**. **Chylomicrons** are assembled in **intestinal mucosal cells** from **dietary lipids** (primarily TAG). Each nascent chylomicron particle has one molecule of **apolipoprotein** (**apo**) **B-48**. They are released from the cells into the lymphatic system and travel to the blood, where they receive **apo C-II** and **apo E** from **HDLs**. Apo C-II activates endothelial lipoprotein lipase (LPL), which degrades the TAG in chylomicrons to fatty acids and glycerol. The **fatty acids** that are released are **stored** (in the **adipose**) or used for **energy** (by the **muscle**). The **glycerol** is metabolized by the **liver**. Patients with a **deficiency** of LPL or **apo C-II** show a dra-



Binding of steroid hormone receptor complex to the HRE of the enhancer region activates the gene promoter causing transcription.

Figure 18.28
Activation of transcription by interaction of steroid hormone–receptor complex with hormone response element (HRE). The receptor contains domains that bind the hormone, DNA, and proteins that relax the DNA. mRNA = messenger RNA.

matic accumulation of chylomicrons in the plasma (**type I hyperlipoproteinemia**, or **familial** LPL **deficiency**) even if fasted. After most of the TAG is removed, apo C-II is returned to the HDL, and the **chylomicron remnant**, carrying most of the **dietary cholesterol**, binds to a **receptor** on the **liver** that recognizes **apo E**. The particle is **endocytosed**, and its contents degraded by **lysosomal enzymes**. Defective uptake of these remnants causes type III hyperlipoproteinemia. **Nascent VLDLs** are produced in the **liver** and are composed predominantly of **TAG**. They contain a single molecule of **apo B-100**. Like nascent chylomicrons, VLDLs receive **apo C-II** and **apo E** from **HDL** in the plasma. The **function** of VLDL is to **carry hepatic TAG** to the **peripheral tissues** where LPL degrades the lipid. Additionally, the VLDL particle receives **cholesteryl esters** from **HDL in exchange for TAG**. This process is accomplished by cholesteryl ester transfer protein (CETP). Eventually, VLDL in the **plasma** is **converted to LDL**, a much smaller, denser particle. Apo C-II and apo E are returned to HDL, but the LDL retains **apo B-100**, which is recognized by **receptors** on **peripheral tissues** and the **liver**. LDLs undergo **receptor-mediated endocytosis**, and their contents are degraded in the **lysosomes**. A **deficiency of functional LDL receptors** causes **type II hyperlipoproteinemia** (**familial hypercholesterolemia**). The endocytosed cholesterol decreases synthesis of HMG CoA reductase (and of **LDL receptors**) through prevention of SREBP-2 binding to the SRE. Some of it can also be esterified by acyl CoA:cholesterol acyltransferase (ACAT) and stored. **HDL** are created by **lipidation of apo A-1** synthesized in the **liver** and **intestine**. They have a number of functions, including: 1) serving as a **circulating reservoir of apo C-II** and **apo E** for chylomicrons and VLDL; 2) removing **unesterified cholesterol** from from peripheral tissues via ABCA1and **esterifying it** using lecithin:cholesterol acyl transferase (LCAT), a liver-synthesized plasma enzyme that is activated by **apo A-1**; and 3) delivering these cholesteryl esters to the liver (**reverse cholesterol transport**) for uptake via scavenger receptor-B1(SR-B1).

Cholesterol is the precursor of all classes of steroid hormones, which include **glucocorticoids**, **mineralocorticoids**, and the **sex hormones** (**androgens**, **estrogens**, and **progestins**). **Synthesis**, using primarily **cytochrome P450** mixed-function oxidases, occurs in the **adrenal cortex** (**cortisol**, **aldosterone**, and **androgens**), **ovaries** and **placenta** (**estrogens** and **progestins**), and **testes** (**testosterone**). The initial and rate-limiting step is the conversion of cholesterol to **pregnenolone** by the side-chain cleavage enzyme P450$_{scc}$. Deficiencies in synthesis lead to **congenital adrenal hyperplasia.** Each steroid hormone diffuses across the plasma membrane of its target cell and binds to a specific **cytosolic** or **nuclear receptor**. These **receptor–ligand complexes** accumulate in the nucleus, dimerize, and **bind** to specific regulatory DNA sequences (**hormone-response elements**) in association with coactivator proteins, thereby causing **promoter activation** and **increased transcription** of targeted genes. In association with corepressors, **transcription is decreased**.

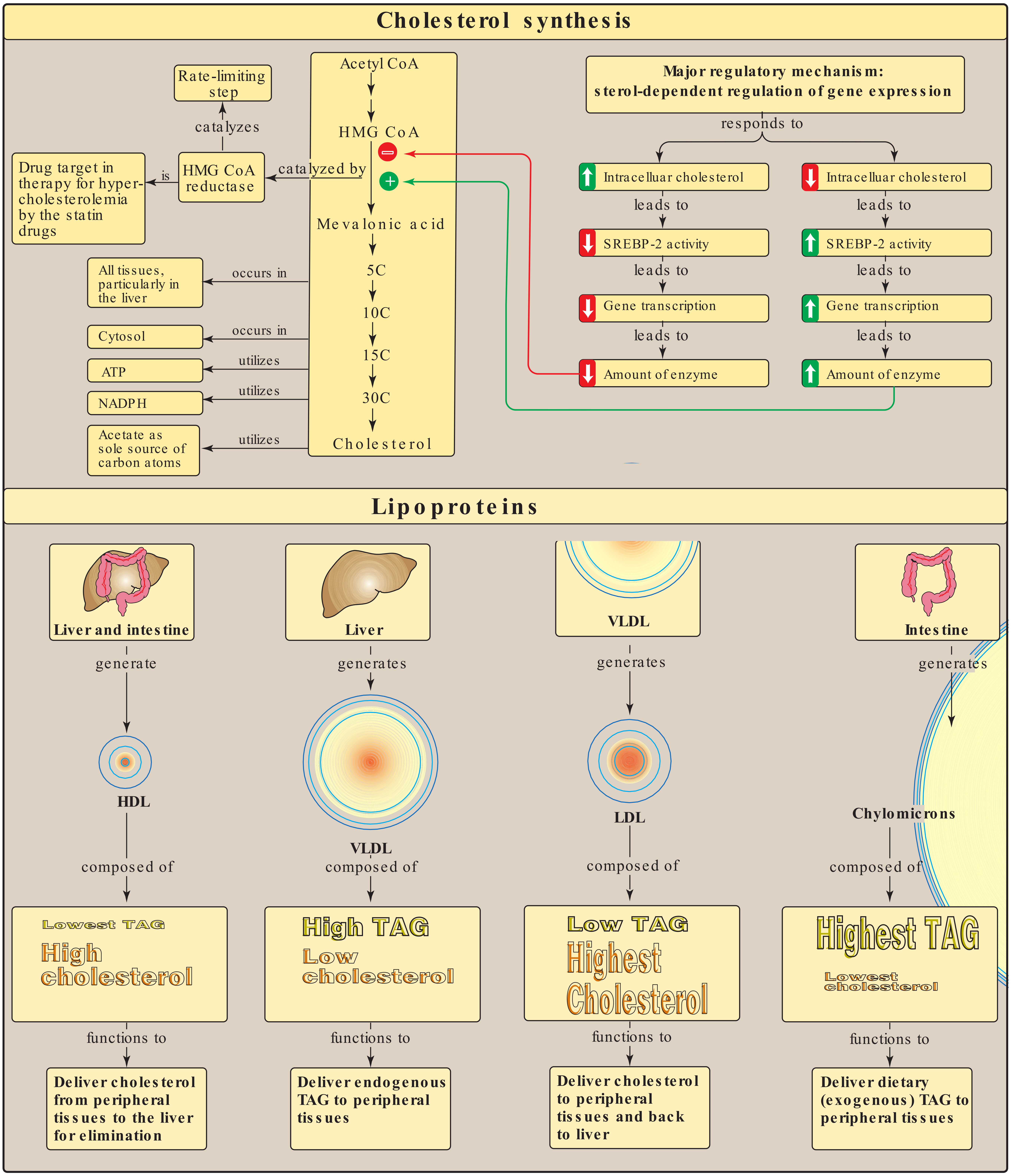

Cholesterol synthesis
Acetyl CoA
HMG CoA
Mevalonic acid
5C
10C
15C
30C
Cholesterol
Rate-limiting step
catalyzes
HMG CoA reductase
catalyzed by
is
Drug target in therapy for hyper-cholesterolemia by the statin drugs
All tissues, particularly in the liver
occurs in
Cytosol
occurs in
ATP
utilizes
NADPH
utilizes
Acetate as sole source of carbon atoms
utilizes
−
+
Major regulatory mechanism: sterol-dependent regulation of gene expression
responds to
Intracellular cholesterol
leads to
SREBP-2 activity
leads to
Gene transcription
leads to
Amount of enzyme
Intracellular cholesterol
leads to
SREBP-2 activity
leads to
Gene transcription
leads to
Amount of enzyme
Lipoproteins
Liver and intestine
generate
HDL
composed of
Lowest TAG
High cholesterol
functions to
Deliver cholesterol from peripheral tissues to the liver for elimination
Liver
generates
VLDL
composed of
High TAG
Low cholesterol
functions to
Deliver endogenous TAG to peripheral tissues
VLDL
generates
LDL
composed of
Low TAG
Highest Cholesterol
functions to
Deliver cholesterol to peripheral tissues and back to liver
Intestine
generates
Chylomicrons
composed of
Highest TAG
Lowest cholesterol
functions to
Deliver dietary (exogenous) TAG to peripheral tissues

Figure 18.29
Concept map for cholesterol and the lipoproteins. HMG CoA = hydroxymethylglutaryl coenzyme A; SREBP = sterol regulatory element–binding protein; HDL = high-density lipoprotein; VLDL = very-low-density lipoprotein; LDL = low-density lipoprotein; TAG = triacylglycerol; NADP(H) = nicotinamide adenine dinucleotide phosphate.

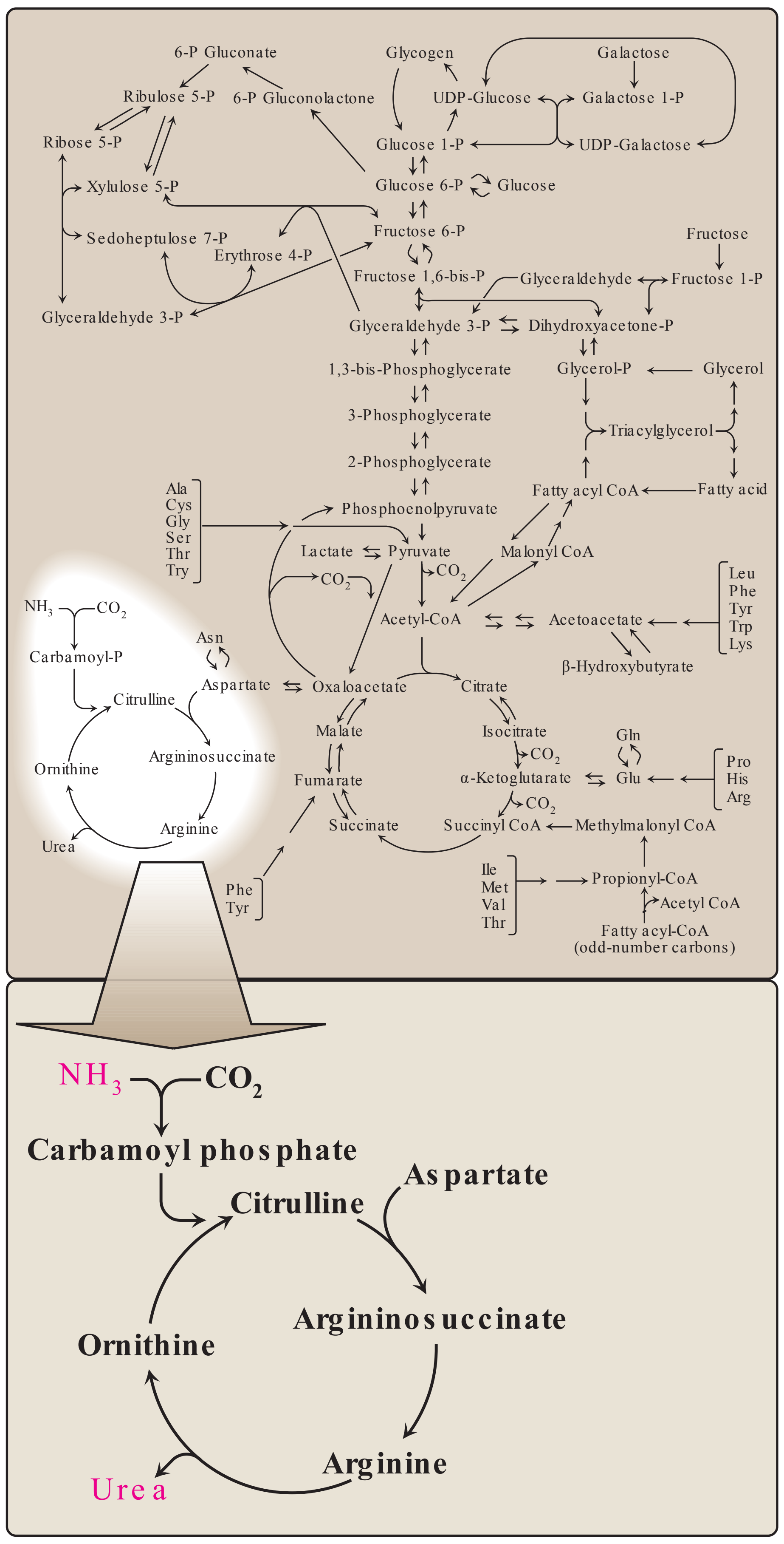
UNIT IV:
Nitrogen Metabolism

Amino Acids: Disposal of Nitrogen

19

I. OVERVIEW

Unlike fats and carbohydrates, amino acids are not stored by the body. That is, no protein exists whose sole function is to maintain a supply of amino acids for future use. Therefore, amino acids must be obtained from the diet, synthesized de novo, or produced from normal protein degradation. Any amino acids in excess of the biosynthetic needs of the cell are rapidly degraded. The first phase of catabolism involves the removal of the α-amino groups (usually by transamination and subsequent oxidative deamination), forming ammonia and the corresponding α-keto acids, the "carbon skeletons" of amino acids. A portion of the free ammonia is excreted in the urine, but most is used in the synthesis of urea (Figure 19.1), which is quantitatively the most important route for disposing of nitrogen from the body. In the second phase of amino acid catabolism, described in Chapter 20, the carbon skeletons of the α-keto acids are converted to common intermediates of energy-producing metabolic pathways. These compounds can be metabolized to CO_2 and water, glucose, fatty acids, or ketone bodies by the central pathways of metabolism described in Chapters 8–13 and 16.



6-P Gluconate
Ribulose 5-P
6-P Gluconolactone
Ribose 5-P
Xylulose 5-P
Sedoheptulose 7-P
Erythrose 4-P
Glyceraldehyde 3-P
Glycogen
UDP-Glucose
Galactose
Galactose 1-P
UDP-Galactose
Glucose 1-P
Glucose 6-P
Glucose
Fructose 6-P
Fructose 1,6-bis-P
Glyceraldehyde
Fructose
Fructose 1-P
Glyceraldehyde 3-P
Dihydroxyacetone-P
1,3-bis-Phosphoglycerate
Glycerol-P
Glycerol
3-Phosphoglycerate
Triacylglycerol
2-Phosphoglycerate
Fatty acyl CoA
Fatty acid
Ala
Cys
Gly
Ser
Thr
Try
Phosphoenolpyruvate
Lactate
Pyruvate
Malonyl CoA
CO2
Acetyl-CoA
Acetoacetate
Leu
Phe
Tyr
Trp
Lys
β-Hydroxybutyrate
Asn
Aspartate
Oxaloacetate
Citrate
Malate
Isocitrate
Gln
Fumarate
α-Ketoglutarate
Glu
Pro
His
Arg
Succinate
Succinyl CoA
Methylmalonyl CoA
Phe
Tyr
Ile
Met
Val
Thr
Propionyl-CoA
Acetyl CoA
Fatty acyl-CoA (odd-number carbons)
NH3
CO2
Carbamoyl phosphate
Citrulline
Aspartate
Argininosuccinate
Ornithine
Arginine
Urea

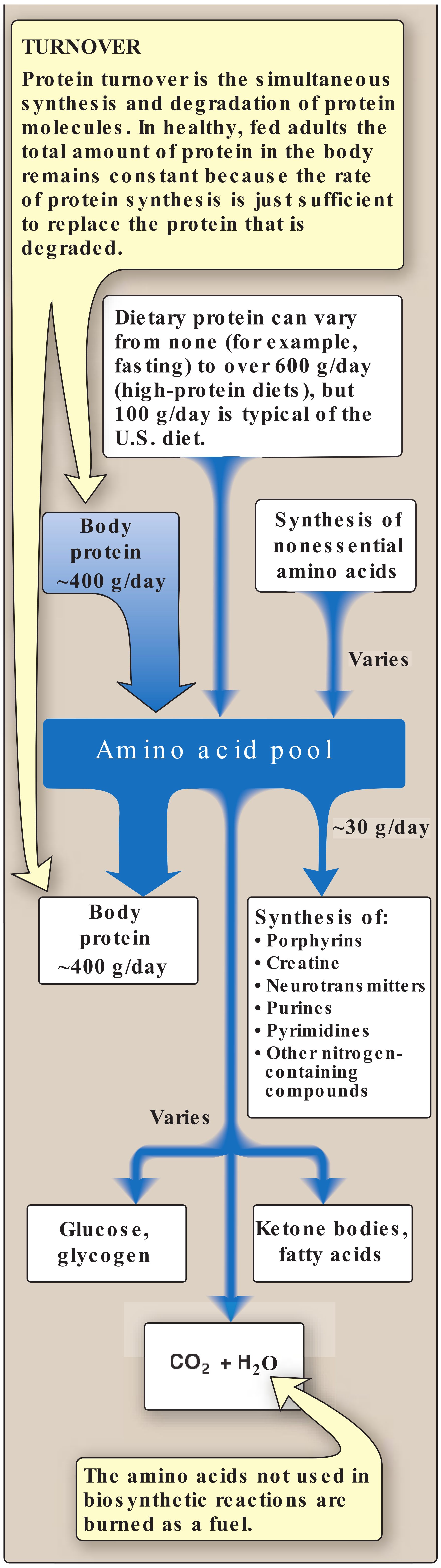
Figure 19.1
Urea cycle shown as part of the essential pathways of energy metabolism. (See Figure 8.2, p. 92, for a more detailed view of intermediary metabolism.)

II. OVERALL NITROGEN METABOLISM

Amino acid catabolism is part of the larger process of the metabolism of nitrogen-containing molecules. Nitrogen enters the body in a variety of compounds present in food, the most important being amino acids contained in dietary protein. Nitrogen leaves the body as urea, ammonia, and other products derived from amino acid metabolism. The role of body proteins in these transformations involves two important concepts: the amino acid pool and protein turnover.

A. Amino acid pool

Free amino acids are present throughout the body, such as in cells, blood, and the extracellular fluids. For the purpose of this discussion, envision all of these amino acids as if they belonged to a single entity, called the amino acid pool. This pool is supplied by three sources: 1) amino acids provided by the degradation of endogenous



TURNOVER

Protein turnover is the simultaneous synthesis and degradation of protein molecules. In healthy, fed adults the total amount of protein in the body remains constant because the rate of protein synthesis is just sufficient to replace the protein that is degraded.

Dietary protein can vary from none (for example, fasting) to over 600 g/day (high-protein diets), but 100 g/day is typical of the U.S. diet.

The amino acids not used in biosynthetic reactions are burned as a fuel.

Figure 19.2
Sources and fates of amino acids.

(body) proteins, most of which are reutilized; 2) amino acids derived from exogenous (dietary) protein; and 3) nonessential amino acids synthesized from simple intermediates of metabolism (Figure 19.2). Conversely, the amino pool is depleted by three routes: 1) synthesis of body protein; 2) consumption of amino acids as precursors of essential nitrogen-containing small molecules; and 3) conversion of amino acids to glucose, glycogen, fatty acids, and ketone bodies, or oxidation to $CO_2 + H_2O$ (see Figure 19.2). Although the amino acid pool is small (comprising about 90–100 g of amino acids) in comparison with the amount of protein in the body (about 12 kg in a 70-kg man), it is conceptually at the center of whole-body nitrogen metabolism.

> In healthy, well-fed individuals, the input to the amino acid pool is balanced by the output. That is, the amount of amino acids contained in the pool is constant. The amino acid pool is said to be in a steady state, and the individual is said to be in nitrogen balance.

B. Protein turnover

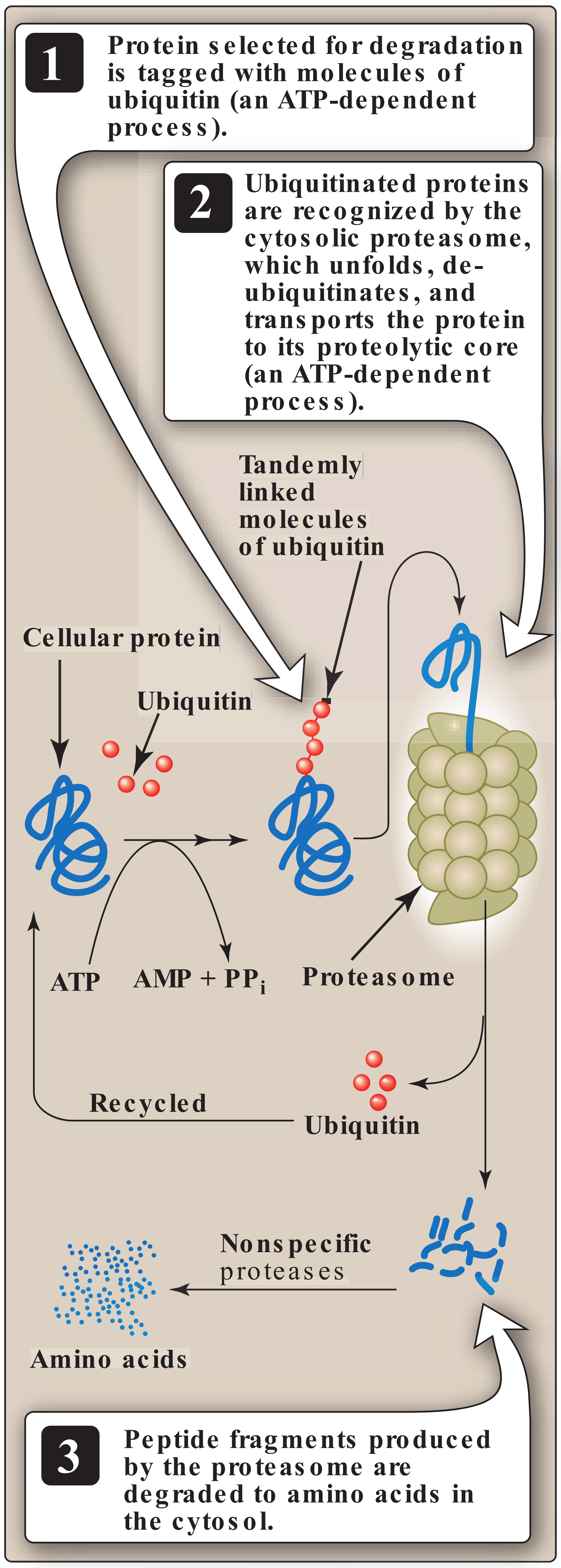
Most proteins in the body are constantly being synthesized and then degraded, permitting the removal of abnormal or unneeded proteins. For many proteins, regulation of synthesis determines the concentration of protein in the cell, with protein degradation assuming a minor role. For other proteins, the rate of synthesis is constitutive (that is, essentially constant), and cellular levels of the protein are controlled by selective degradation.

1. **Rate of turnover:** In healthy adults, the total amount of protein in the body remains constant because the rate of protein synthesis is just sufficient to replace the protein that is degraded. This process, called protein turnover, leads to the hydrolysis and resynthesis of 300–400 g of body protein each day. The rate of protein turnover varies widely for individual proteins. Short-lived proteins (for example, many regulatory proteins and misfolded proteins) are rapidly degraded, having half-lives measured in minutes or hours. Long-lived proteins, with half-lives of days to weeks, constitute the majority of proteins in the cell. Structural proteins, such as collagen, are metabolically stable and have half-lives measured in months or years.

2. **Protein degradation:** There are two major enzyme systems responsible for degrading proteins: the adenosine triphosphate (ATP)-dependent ubiquitin-proteasome system of the cytosol, and the ATP-independent degradative enzyme system of the lysosomes. Proteasomes selectively degrade damaged or short-lived proteins. Lysosomes use acid hydrolases (see p. 162) to nonselectively degrade intracellular proteins ("autophagy") and extracellular proteins ("heterophagy"), such as plasma proteins, that are taken into the cell by endocytosis.

a. **Ubiquitin–proteasome proteolytic pathway:** Proteins selected for degradation by the cytosolic ubiquitin-proteasome system are first modified by the covalent attachment of ubiquitin (Ub), a small, globular, nonenzymic protein that is highly conserved across eukaryotic species. Ubiquitination of the target substrate occurs through isopeptide linkage of the α-carboxyl group of the C-terminal glycine of Ub to the ε-amino group of a lysine on the protein substrate by a three-step, enzyme-catalyzed, ATP-dependent process. [Note: Enzyme 1 (E1, or activating enzyme) activates Ub, which is then transferred to E2 (conjugating enzyme). E3 (a ligase) identifies the protein to be degraded and interacts with E2-Ub.] The consecutive addition of four or more Ub molecules to the target protein generates a polyubiquitin chain. Proteins tagged with Ub are recognized by a large, barrel-shaped, macromolecular, proteolytic complex called a proteasome (Figure 19.3). The proteasome unfolds, deubiquitinates, and cuts the target protein into fragments that are then further degraded by cytosolic proteases to amino acids, which enter the amino acid pool. Ub is recycled. It is noteworthy that the selective degradation of proteins by the ubiquitin-proteosome complex (unlike simple hydrolysis by proteolytic enzymes) requires energy in the form of ATP.

b. **Chemical signals for protein degradation:** Because proteins have different half-lives, it is clear that protein degradation cannot be random but, rather, is influenced by some structural aspect of the protein. For example, some proteins that have been chemically altered by oxidation or tagged with ubiquitin are preferentially degraded. The half-life of a protein is also influenced by the amino (N)-terminal residue. For example, proteins that have serine as the N-terminal amino acid are long-lived, with a half-life of more than 20 hours, whereas those with aspartate at their N-terminus have a half-life of only 3 minutes. Additionally, proteins rich in sequences containing proline, glutamate, serine, and threonine (called PEST sequences after the one-letter designations for these amino acids) are rapidly degraded and, therefore, have short half-lives.



1 Protein selected for degradation is tagged with molecules of ubiquitin (an ATP-dependent process).

2 Ubiquitinated proteins are recognized by the cytosolic proteasome, which unfolds, deubiquitinates, and transports the protein to its proteolytic core (an ATP-dependent process).

3 Peptide fragments produced by the proteasome are degraded to amino acids in the cytosol.

Figure 19.3
The ubiquitin–proteasome degradation pathway of proteins. AMP = adenosine monophosphate; PP_i = pyrophosphate.

III. DIGESTION OF DIETARY PROTEINS

Most of the nitrogen in the diet is consumed in the form of protein, typically amounting to 70–100 g/day in the American diet (see Figure 19.2). Proteins are generally too large to be absorbed by the intestine. [Note: An example of an exception to this rule is that newborns can take up maternal antibodies in breast milk.] They must, therefore, be hydrolyzed to yield di- and tripeptides as well as individual amino acids, which can be absorbed. Proteolytic enzymes responsible for degrading proteins are produced by three different organs: the stomach, the pancreas, and the small intestine (Figure 19.4).

A. Digestion by gastric secretion

The digestion of proteins begins in the stomach, which secretes gastric juice, a unique solution containing hydrochloric acid and the proenzyme pepsinogen.

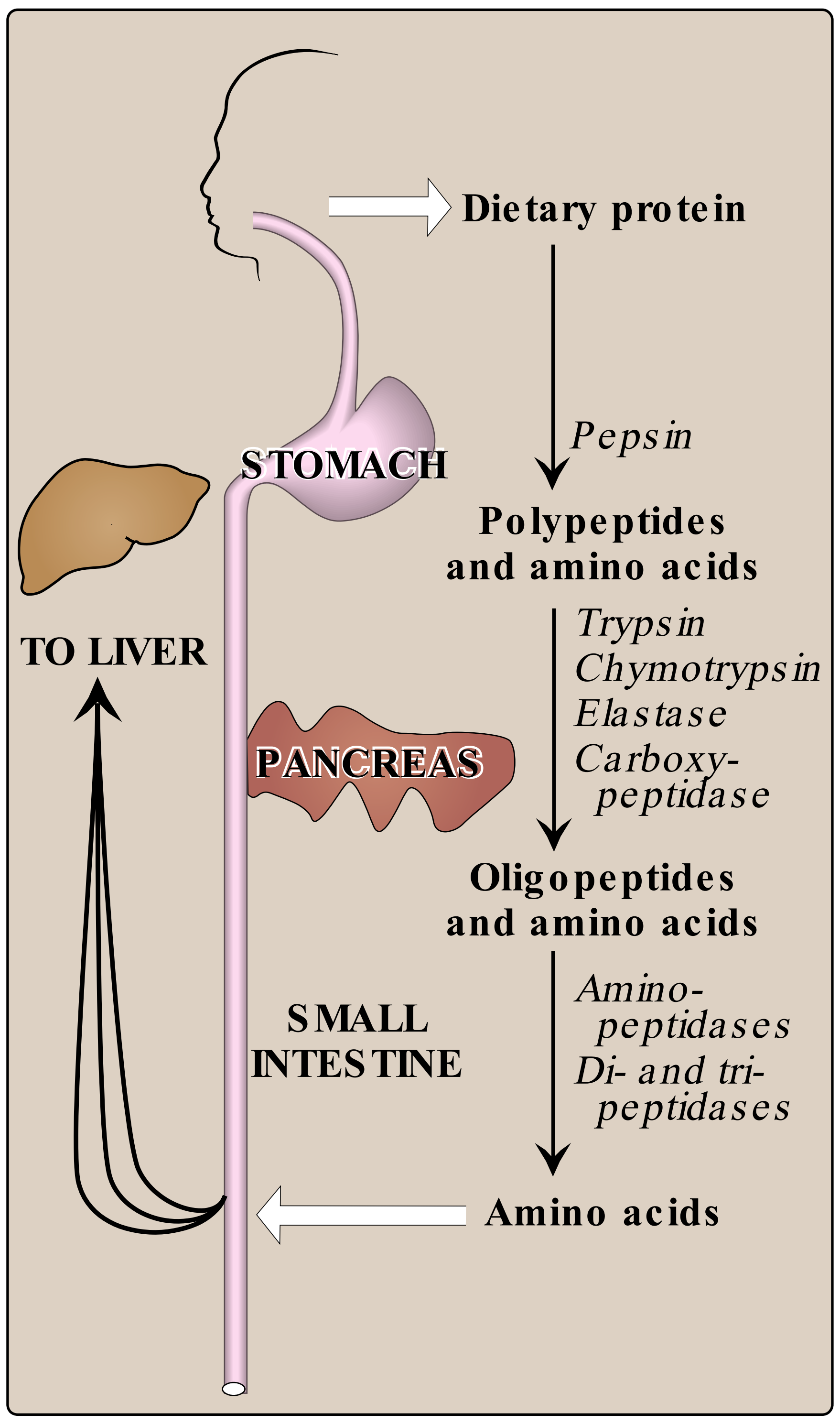


Figure 19.4
Digestion of dietary proteins by the proteolytic enzymes of the gastrointestinal tract.

1. **Hydrochloric acid:** Stomach acid is too dilute (pH 2–3) to hydrolyze proteins. The acid, secreted by the parietal cells of the stomach, functions instead to kill some bacteria and to denature proteins, thereby making them more susceptible to subsequent hydrolysis by proteases.

2. **Pepsin:** This acid-stable endopeptidase is secreted by the chief cells of the stomach as an inactive zymogen (or proenzyme), pepsinogen. [Note: In general, zymogens contain extra amino acids in their sequences that prevent them from being catalytically active. Removal of these amino acids permits the proper folding required for an active enzyme.] Pepsinogen is activated to pepsin, either by hydrochloric acid or autocatalytically by pepsin molecules that have already been activated. Pepsin releases peptides and a few free amino acids from dietary proteins.

B. Digestion by pancreatic enzymes

On entering the small intestine, large polypeptides produced in the stomach by the action of pepsin are further cleaved to oligopeptides and amino acids by a group of pancreatic proteases that include both endopeptidases (cleave within) and exopeptidases (cut at an end). [Note: Bicarbonate (HCO_3^-), also secreted by the pancreas, raises the pH.]

1. **Specificity:** Each of these enzymes has a different specificity for the amino acid R-groups adjacent to the susceptible peptide bond (Figure 19.5). For example, trypsin cleaves only when the carbonyl group of the peptide bond is contributed by arginine or lysine. These enzymes, like pepsin described above, are synthesized and secreted as inactive zymogens.

2. **Release of zymogens:** The release and activation of the pancreatic zymogens is mediated by the secretion of cholecystokinin and secretin, two polypeptide hormones of the digestive tract (see p. 176).

3. **Activation of zymogens:** Enteropeptidase (formerly called enterokinase), an enzyme synthesized by and present on the luminal surface of intestinal mucosal cells of the brush border membrane, converts the pancreatic zymogen trypsinogen to trypsin by removal of a hexapeptide from the N-terminus of trypsinogen. Trypsin subsequently converts other trypsinogen molecules to trypsin by cleaving a limited number of specific peptide bonds in the zymogen. Enteropeptidase, thus, unleashes a cascade of proteolytic activity because trypsin is the common activator of all the pancreatic zymogens (see Figure 19.5).

4. **Abnormalities in protein digestion:** In individuals with a deficiency in pancreatic secretion (for example, due to chronic pancreatitis, cystic fibrosis, or surgical removal of the pancreas), the digestion and absorption of fat and protein are incomplete. This results in the abnormal appearance of lipids in the feces (a condition called steatorrhea; see p. 177) as well as undigested protein.

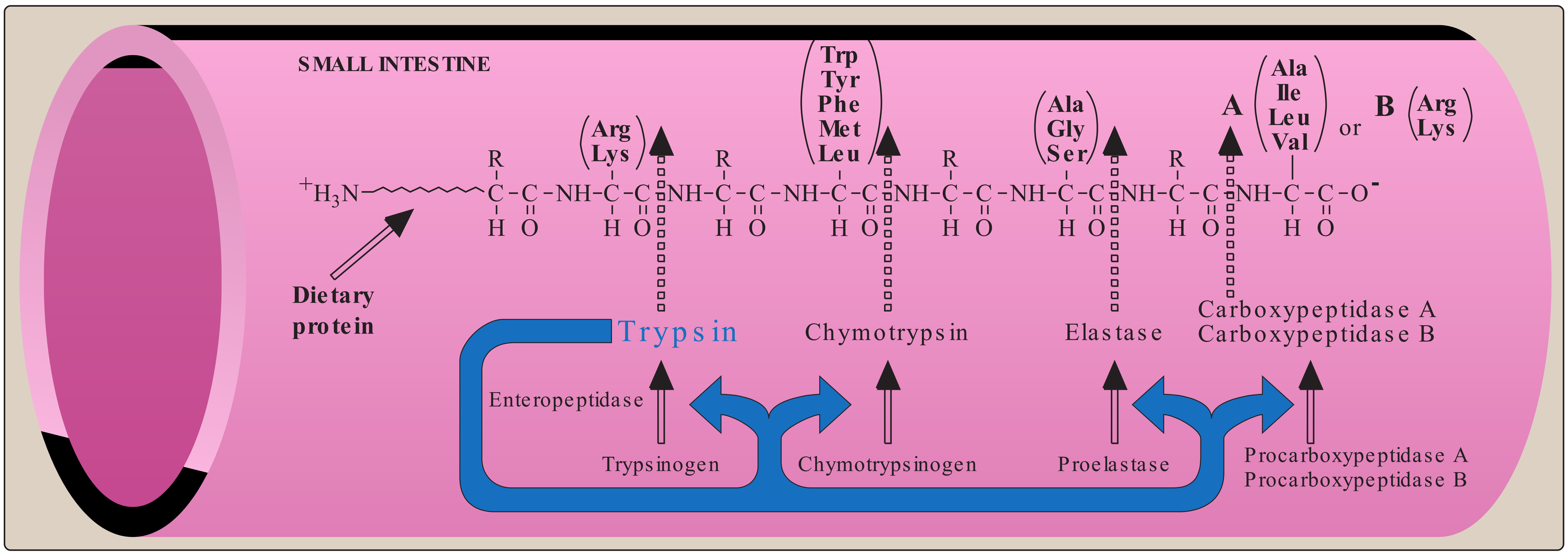

SMALL INTESTINE
Dietary protein
Trp Tyr Phe Met Leu
Arg Lys
Ala Gly Ser
A
Ala Ile Leu Val
or
B
Arg Lys
Trypsin
Chymotrypsin
Elastase
Carboxypeptidase A
Carboxypeptidase B
Enteropeptidase
Trypsinogen
Chymotrypsinogen
Proelastase
Procarboxypeptidase A
Procarboxypeptidase B

Figure 19.5
Cleavage of dietary protein in the small intestine by pancreatic proteases. The peptide bonds susceptible to hydrolysis are shown for each of the five major pancreatic proteases. [Note: The first three are serine endopeptidases, whereas the last two are exopeptidases. Each is produced from an inactive zymogen.]

Celiac disease (celiac sprue) is a disease of malabsorption resulting from immune-mediated damage to the small intestine in response to ingestion of gluten (or gliadin produced from gluten), a protein found in wheat, barley and rye.

C. Digestion of oligopeptides by enzymes of the small intestine

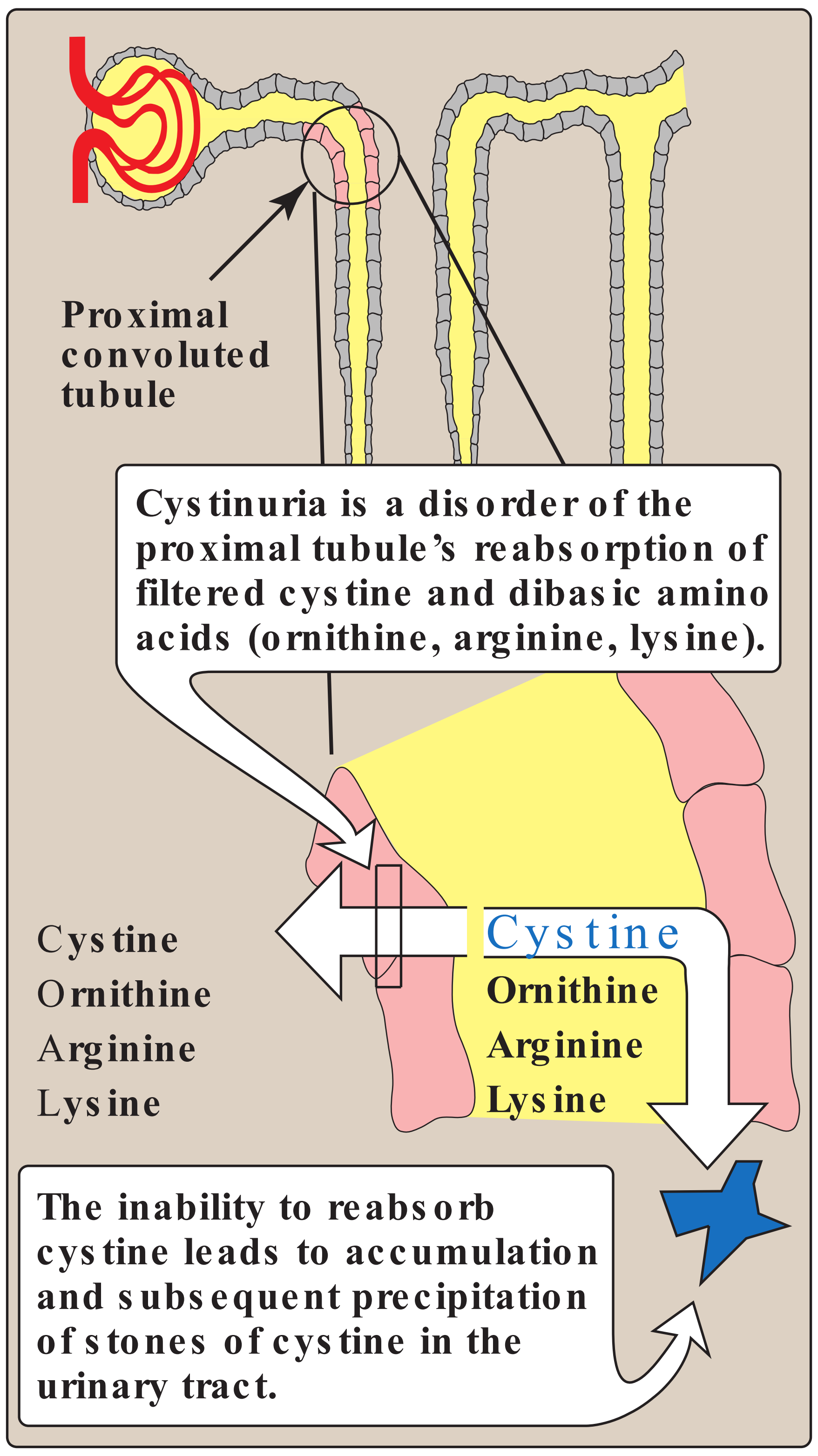
The luminal surface of the intestine contains aminopeptidase, an exopeptidase that repeatedly cleaves the N-terminal residue from oligopeptides to produce even smaller peptides and free amino acids.

D. Absorption of amino acids and small peptides

Free amino acids are taken into the enterocytes by a sodium-linked secondary transport system of the apical membrane. Di- and tripeptides, however, are taken up by a proton-linked transport system. The peptides are hydrolyzed in the cytosol to amino acids that are released into the portal system by facilitated diffusion. Therefore, only free amino acids are found in the portal vein after a meal containing protein. These amino acids are either metabolized by the liver or released into the general circulation. [Note: Branched-chain amino acids are important examples of amino acids that are not metabolized by the liver but, instead, are sent from the liver primarily to muscle via the blood.]

IV. TRANSPORT OF AMINO ACIDS INTO CELLS

The concentration of free amino acids in the extracellular fluids is significantly lower than that within the cells of the body. This concentration gradient is maintained because active transport systems, driven by the hydrolysis of ATP, are required for movement of amino acids from the extracellular space into cells. At least seven different transport


Proximal convoluted tubule
Cystine
Ornithine
Arginine
Lysine
Cystine
Ornithine
Arginine
Lysine

Cystinuria is a disorder of the proximal tubule's reabsorption of filtered cystine and dibasic amino acids (ornithine, arginine, lysine).

The inability to reabsorb cystine leads to accumulation and subsequent precipitation of stones of cystine in the urinary tract.

Figure 19.6
Genetic defect seen in cystinuria. [Note: Cystinuria is distinct from cystinosis, a rare defect in the transport of cystine out of lysosomes that results in the formation of cystine crystals within the lysosome and tissue damage.]

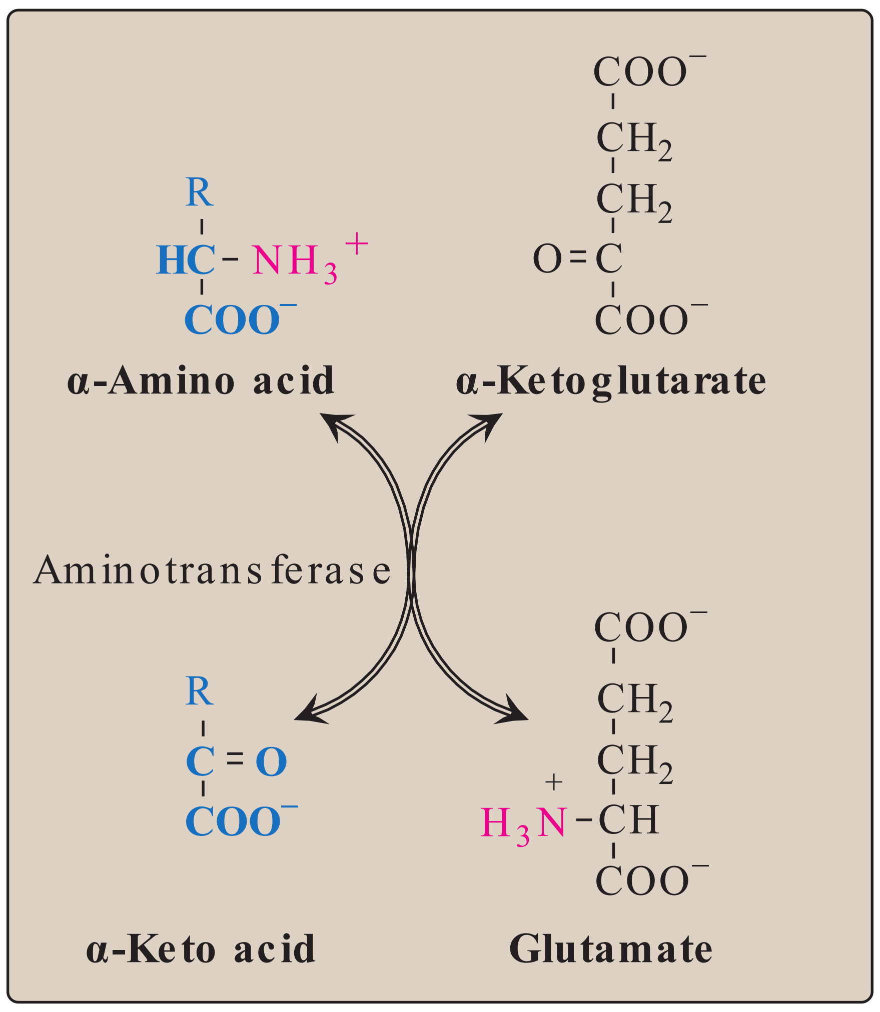


Figure 19.7
Aminotransferase reaction using α-ketoglutarate as the amino group acceptor.

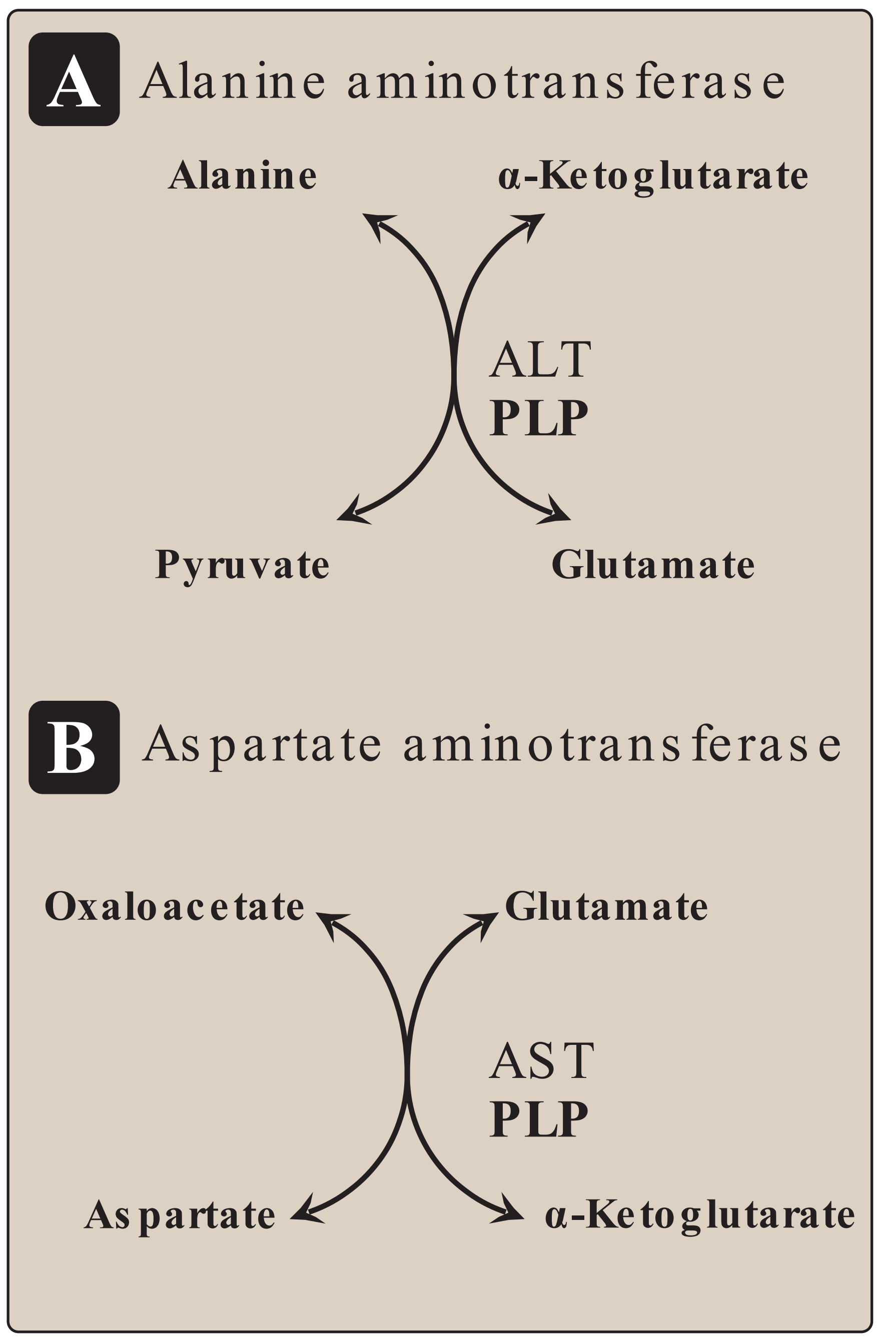


Figure 19.8
Reactions catalyzed during amino acid catabolism. A. Alanine aminotransferase (ALT). B. Aspartate aminotransferase (AST). PLP = pyridoxal phosphate (see p. 251).

systems are known that have overlapping specificities for different amino acids. Because the small intestine and the proximal tubule of the kidney have common transport systems for amino acid uptake, a defect in any one of these systems results in an inability to absorb particular amino acids into the gut and into the kidney tubules. For example, one system is responsible for the uptake of cystine and the dibasic amino acids, ornithine, arginine, and lysine (represented as "COAL"). In the inherited disorder cystinuria, this carrier system is defective, and all four amino acids appear in the urine (Figure 19.6). Cystinuria occurs at a frequency of 1 in 7,000 individuals, making it one of the most common inherited diseases and the most common genetic error of amino acid transport. The disease expresses itself clinically by the precipitation of cystine to form kidney stones (calculi), which can block the urinary tract. Oral hydration is an important part of treatment for this disorder. [Note: Defects in the transport of tryptophan can result in Hartnup disorder and pellagra-like (see p. 380) dermatologic and neurologic symptoms.]

V. REMOVAL OF NITROGEN FROM AMINO ACIDS

The presence of the α-amino group keeps amino acids safely locked away from oxidative breakdown. Removing the α-amino group is essential for producing energy from any amino acid and is an obligatory step in the catabolism of all amino acids. Once removed, this nitrogen can be incorporated into other compounds or excreted as urea, with the carbon skeletons being metabolized. This section describes transamination and oxidative deamination, reactions that ultimately provide ammonia and aspartate, the two sources of urea nitrogen (see p. 253).

A. Transamination: the funneling of amino groups to glutamate

The first step in the catabolism of most amino acids is the transfer of their α-amino group to α-ketoglutarate (Figure 19.7), producing an α-keto acid (derived from the original amino acid) and glutamate. α-Ketoglutarate plays a pivotal role in amino acid metabolism by accepting the amino groups from most amino acids, thereby becoming glutamate. Glutamate produced by transamination can be oxidatively deaminated (see below) or used as an amino group donor in the synthesis of nonessential amino acids. This transfer of amino groups from one carbon skeleton to another is catalyzed by a family of enzymes called aminotransferases (also called transaminases). These enzymes are found in the cytosol and mitochondria of cells throughout the body. All amino acids, with the exception of lysine and threonine, participate in transamination at some point in their catabolism. [Note: These two amino acids lose their α-amino groups by deamination (see pp. 265–266).]

1. **Substrate specificity of aminotransferases:** Each aminotransferase is specific for one or, at most, a few amino group donors. Aminotransferases are named after the specific amino group donor, because the acceptor of the amino group is almost always α-ketoglutarate. Two important aminotransferase reactions are catalyzed by alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as shown in Figure 19.8).

   a. **Alanine aminotransferase:** ALT is present in many tissues. The enzyme catalyzes the transfer of the amino group of alanine to α-ketoglutarate, resulting in the formation of pyruvate and glutamate. The reaction is readily reversible. However, during amino acid catabolism, this enzyme (like most aminotransferases) functions in the direction of glutamate synthesis. [Note: Glutamate, in effect, acts as a "collector" of nitrogen from most amino acids.]

b. **Aspartate aminotransferase:** AST is an exception to the rule that aminotransferases funnel amino groups to form glutamate. During amino acid catabolism, AST transfers amino groups from glutamate to oxaloacetate, forming aspartate, which is used as a source of nitrogen in the urea cycle (see p. 253). Like other transaminations, the AST reaction is reversible.

2. **Mechanism of action of aminotransferases:** All aminotransferases require the coenzyme pyridoxal phosphate (a derivative of vitamin B_6; see p. 378), which is covalently linked to the ε-amino group of a specific lysine residue at the active site of the enzyme. Aminotransferases act by transferring the amino group of an amino acid to the pyridoxal part of the coenzyme to generate pyridoxamine phosphate. The pyridoxamine form of the coenzyme then reacts with an α-keto acid to form an amino acid, at the same time regenerating the original aldehyde form of the coenzyme. Figure 19.9 shows these two component reactions for the reaction catalyzed by AST.

3. **Equilibrium of transamination reactions:** For most transamination reactions, the equilibrium constant is near 1. This allows the reaction to function in both amino acid degradation through removal of α-amino groups (for example, after consumption of a protein-rich meal) and biosynthesis of nonessential amino acids through addition of amino groups to the carbon skeletons of α-keto acids (for example, when the supply of amino acids from the diet is not adequate to meet the synthetic needs of cells).

4. **Diagnostic value of plasma aminotransferases:** Aminotransferases are normally intracellular enzymes, with the low levels found in the plasma representing the release of cellular contents during normal cell turnover. Elevated plasma levels of aminotransferases indicate damage to cells rich in these enzymes. For example, physical trauma or a disease process can cause cell lysis, resulting in release of intracellular enzymes into the blood. Two aminotransferases, AST and ALT, are of particular diagnostic value when they are found in the plasma.

a. **Liver disease:** Plasma AST and ALT are elevated in nearly all liver diseases but are particularly high in conditions that cause extensive cell necrosis, such as severe viral hepatitis, toxic injury, and prolonged circulatory collapse. ALT is more specific than AST for liver disease, but the latter is more sensitive because the liver contains larger amounts of AST. Serial measurements of AST and ALT (so-called "liver function tests") are often useful in determining the course of liver damage. Figure 19.10 shows the early release of ALT into the serum, following ingestion of a liver toxin. [Note: Elevated serum bilirubin results from hepatocellular damage that decreases the hepatic conjugation and excretion of bilirubin (see p. 284).]

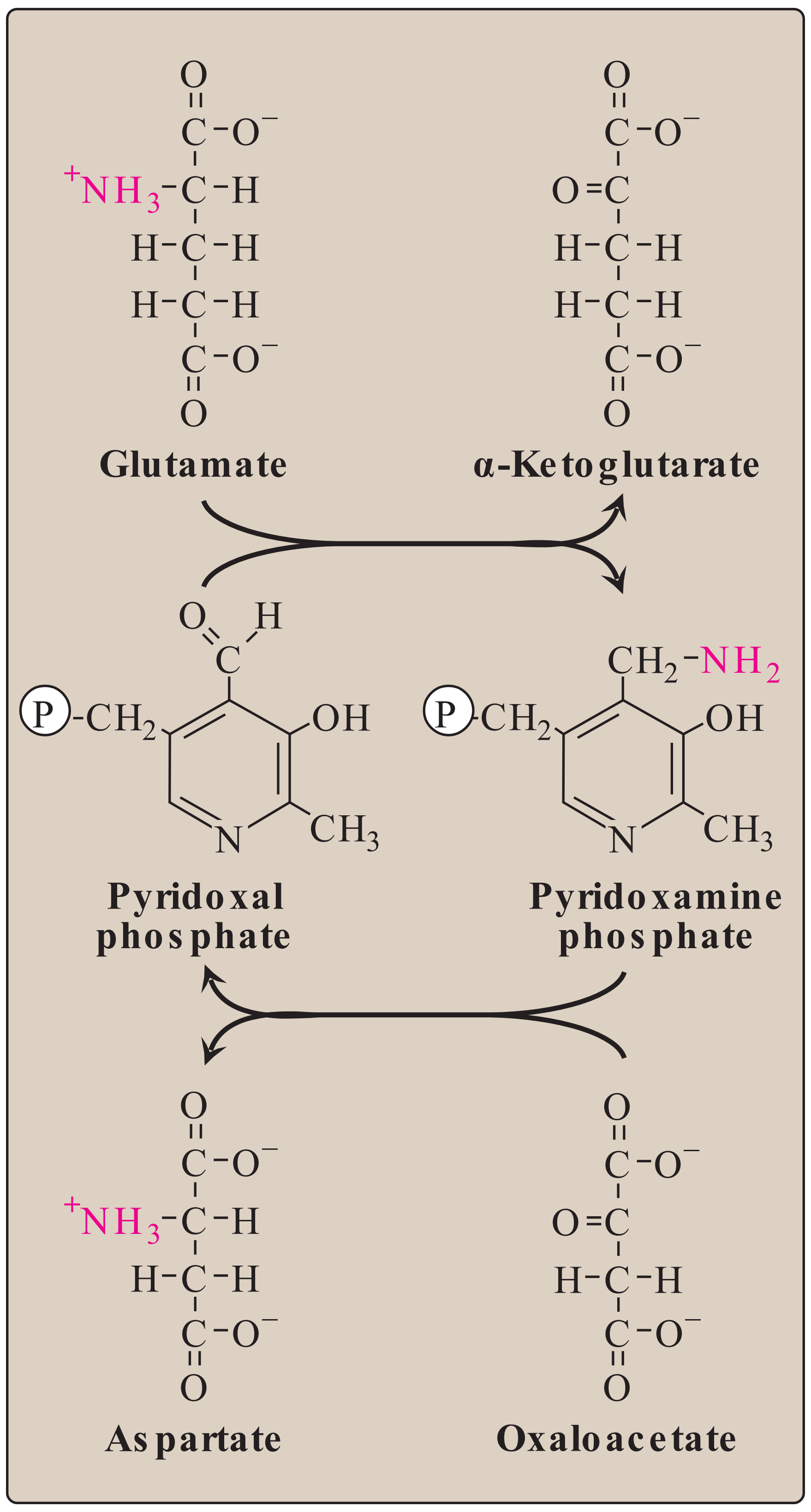


Figure 19.9
Cyclic interconversion of pyridoxal phosphate and pyridoxamine phosphate during the aspartate aminotransferase reaction. Ⓟ = phosphate group.

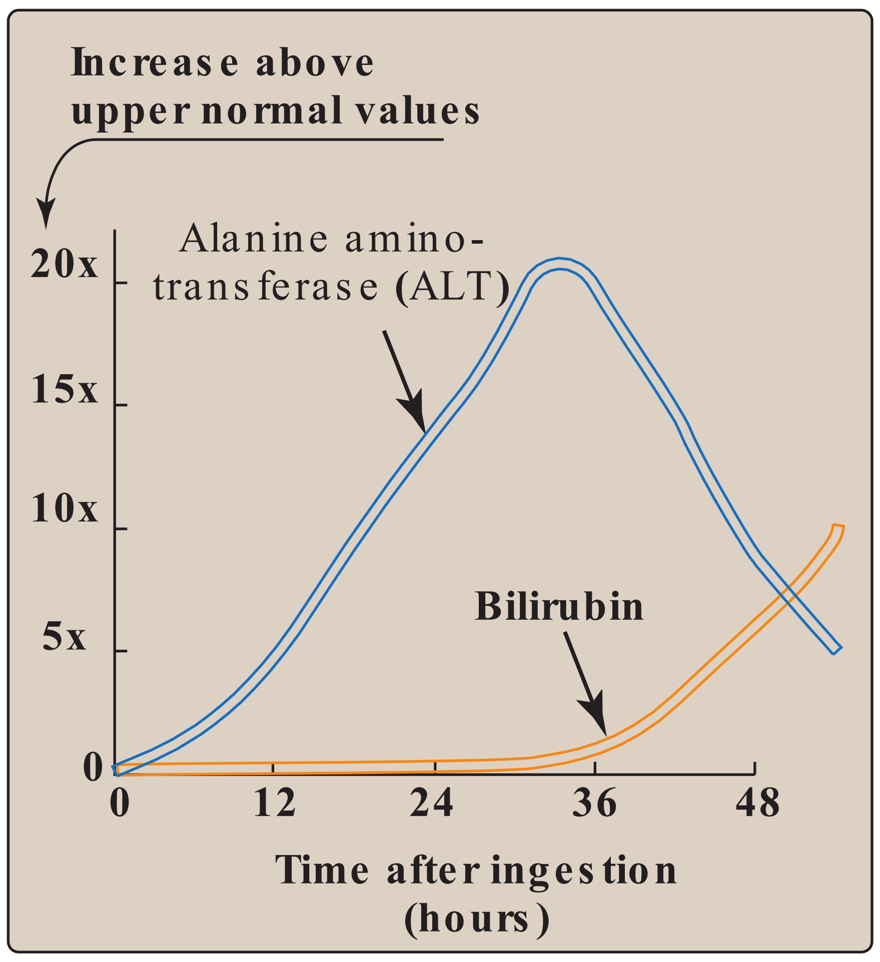


Figure 19.10
Pattern of serum ALT and bilirubin in the plasma, following poisoning with the toxic mushroom Amanita phalloides.

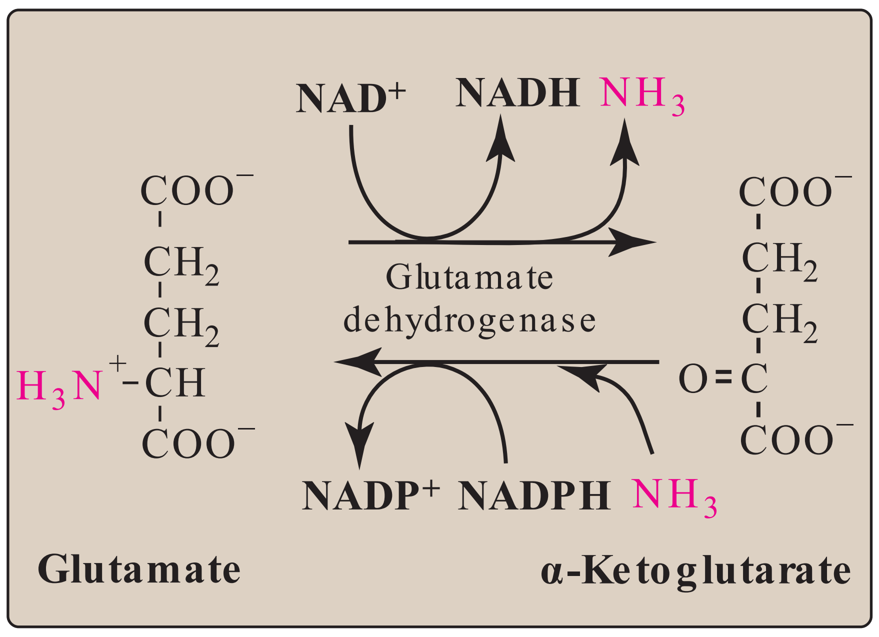

NAD+
NADH
NH3
COO−
CH2
CH2
H3N+-CH
COO−
Glutamate dehydrogenase
COO−
CH2
CH2
O=C
COO−
NADP+
NADPH
NH3
Glutamate
α-Ketoglutarate

Figure 19.11
Oxidative deamination by glutamate dehydrogenase. [Note: The enzyme is unusual in that it uses both NAD^+ (nicotinamide adenine dinucleotide) and NADPH (nicotinamide adenine dinucleotide phosphate).]

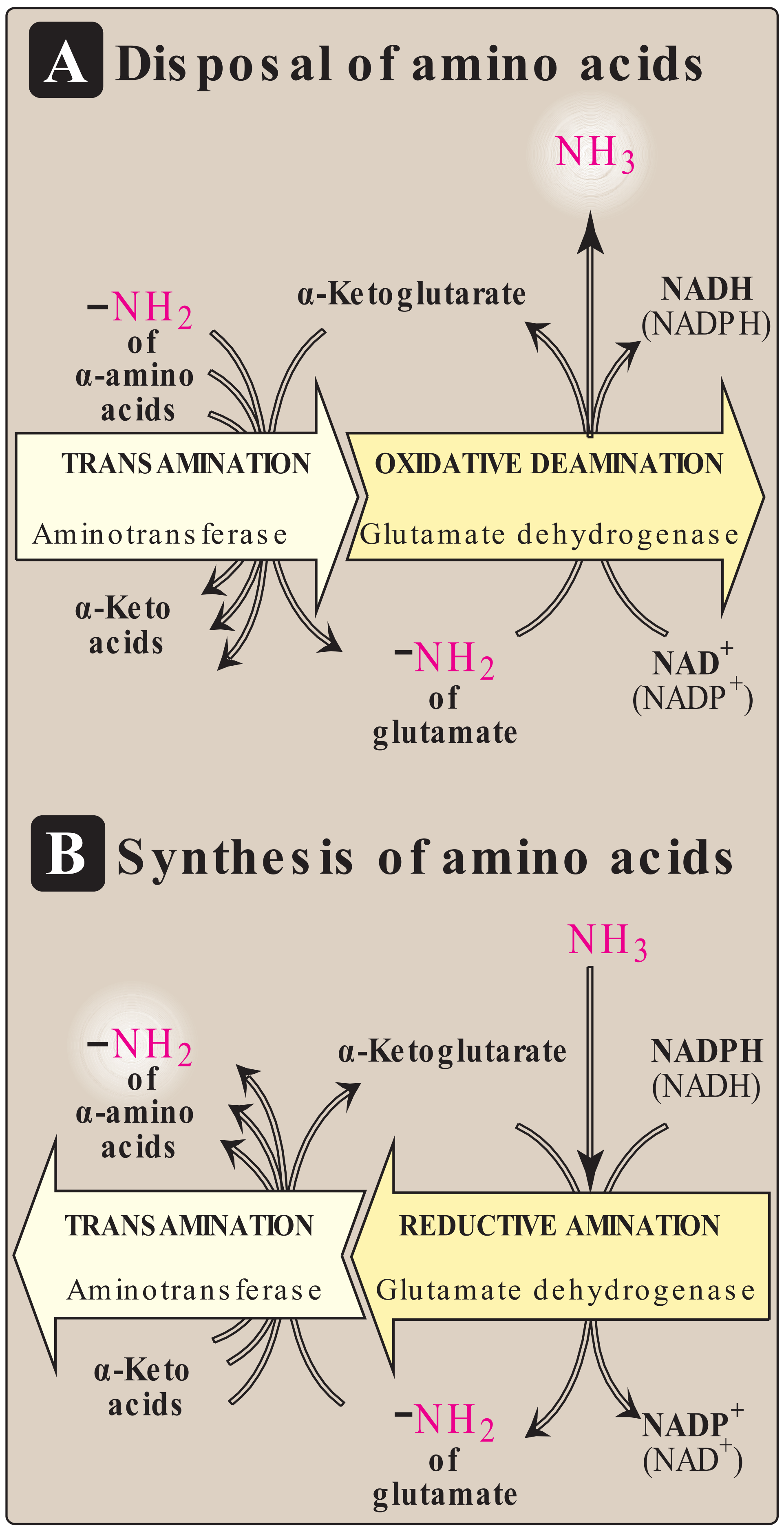

A Disposal of amino acids
NH3
−NH2 of α-amino acids
α-Ketoglutarate
NADH (NADPH)
TRANSAMINATION
Aminotransferase
OXIDATIVE DEAMINATION
Glutamate dehydrogenase
α-Keto acids
−NH2 of glutamate
NAD+ (NADP+)
B Synthesis of amino acids
NH3
−NH2 of α-amino acids
α-Ketoglutarate
NADPH (NADH)
TRANSAMINATION
Aminotransferase
REDUCTIVE AMINATION
Glutamate dehydrogenase
α-Keto acids
−NH2 of glutamate
NADP+ (NAD+)

Figure 19.12
Combined actions of aminotransferase and glutamate dehydrogenase reactions. [Note: Reductive amination occurs only when ammonia (NH_3) level is high.] NAD(H) = nicotinamide adenine dinucleotide; NADP(H) = nicotinamide adenine dinucleotide phosphate.

b. Nonhepatic disease: Aminotransferases may be elevated in nonhepatic diseases such as those that cause damage to cardiac or skeletal muscle. However, these disorders can usually be distinguished clinically from liver disease.

B. Oxidative deamination of amino acids

In contrast to transamination reactions that transfer amino groups, oxidative deamination reactions result in the liberation of the amino group as free ammonia (Figure 19.11). These reactions occur primarily in the liver and kidney. They provide α-keto acids that can enter the central pathways of energy metabolism and ammonia, which is a source of nitrogen in hepatic urea synthesis. [Note: Ammonia exists primarily as ammonium (NH_4^+) in aqueous solution, but it is the un-ionized form (NH_3) that crosses membranes.]

1. **Glutamate dehydrogenase:** As described above, the amino groups of most amino acids are ultimately funneled to glutamate by means of transamination with α-ketoglutarate. Glutamate is unique in that it is the only amino acid that undergoes rapid oxidative deamination, a reaction catalyzed by glutamate dehydrogenase (see Figure 19.11). Therefore, the sequential action of transamination (resulting in the transfer of amino groups from most amino acids to α-ketoglutarate to produce glutamate) and the oxidative deamination of that glutamate (regenerating α-ketoglutarate) provide a pathway whereby the amino groups of most amino acids can be released as ammonia.

   a. Coenzymes: Glutamate dehydrogenase, a mitochondrial enzyme, is unusual in that it can use either nicotinamide adenine dinucleotide (NAD^+) or its phosphorylated reduced form (NADPH) as a coenzyme (see Figure 19.11). NAD^+ is used primarily in oxidative deamination (the simultaneous loss of ammonia coupled with the oxidation of the carbon skeleton, as shown in Figure 19.12A), and NADPH is used in reductive amination (the simultaneous gain of ammonia coupled with the reduction of the carbon skeleton, as shown in Figure 19.12B).

   b. Direction of reactions: The direction of the reaction depends on the relative concentrations of glutamate, α-ketoglutarate, and ammonia and the ratio of oxidized to reduced coenzymes. For example, after ingestion of a meal containing protein, glutamate levels in the liver are elevated, and the reaction proceeds in the direction of amino acid degradation and the formation of ammonia (see Figure 19.12A). High ammonia levels are required to drive the reaction to glutamate synthesis.

   c. Allosteric regulators: Guanosine triphosphate is an allosteric inhibitor of glutamate dehydrogenase, whereas adenosine diphosphate (ADP) is an activator. Therefore, when energy levels are low in the cell, amino acid degradation by glutamate dehydrogenase is high, facilitating energy production from the carbon skeletons derived from amino acids.

2. **D-Amino acid oxidase:** D-Amino acids (see p. 5) are found in plants and in the cell walls of microorganisms but are not used in the synthesis of mammalian proteins. D-Amino acids are, however, present in the diet and are efficiently metabolized by the kidney and liver. D-Amino acid oxidase (DAO) is a flavin adenine dinucleotide–

dependent peroxisomal enzyme that catalyzes the oxidative deamination of these amino acid isomers, thereby producing α-keto acids, ammonia, and hydrogen peroxide. The α-keto acids can enter the general pathways of amino acid metabolism and be reaminated to L-isomers or catabolized for energy. [Note: DAO degrades D-serine, the isomeric form of serine that modulates N-methyl-D-aspartate (NMDA)-type glutamate receptors. Increased DAO activity has been linked to increased susceptibility to schizophrenia.] L-amino acid oxidases are known, but their physiologic significance is unclear.

C. Transport of ammonia to the liver

Two mechanisms are available in humans for the transport of ammonia from the peripheral tissues to the liver for its ultimate conversion to urea. Both are important in, but not exclusive to, skeletal muscle. The first uses glutamine synthetase to combine ammonia with glutamate to form glutamine, a nontoxic transport form of ammonia (Figure 19.13). The glutamine is transported in the blood to the liver where it is cleaved by glutaminase to produce glutamate and free ammonia (see p. 256). The ammonia is converted to urea. The second transport mechanism involves the formation of alanine by the transamination of pyruvate produced from both aerobic glycolysis and metabolism of the succinyl coenzyme A (CoA) generated by the catabolism of the branched-chain amino acids isoleucine and valine. Alanine is transported by the blood to the liver, where it is converted to pyruvate, again by transamination. The pyruvate is used to synthesize glucose, which can enter the blood and be used by muscle, a pathway called the glucose–alanine cycle.

VI. UREA CYCLE

Urea ($H_2N\overset{\overset{O}{\|}}{C}NH_2$) is the major disposal form of amino groups derived from amino acids and accounts for about 90% of the nitrogen-containing components of urine. One nitrogen of the urea molecule is supplied by free ammonia and the other nitrogen by aspartate. [Note: Glutamate is the immediate precursor of both ammonia (through oxidative deamination by glutamate dehydrogenase) and aspartate nitrogen (through transamination of oxaloacetate by AST).] The carbon and oxygen of urea are derived from CO_2 (as HCO_3^-). Urea is produced by the liver and then is transported in the blood to the kidneys for excretion in the urine.

A. Reactions of the cycle

The first two reactions leading to the synthesis of urea occur in the mitochondrial matrix, whereas the remaining cycle enzymes are located in the cytosol (Figure 19.14). [Note: Gluconeogenesis (see p. 117) and heme synthesis (see p. 278) also involve both the mitochondrial matrix and the cytosol.]

1. **Formation of carbamoyl phosphate:** Formation of carbamoyl phosphate by carbamoyl phosphate synthetase I (CPS I) is driven by cleavage of two molecules of ATP. Ammonia incorporated into carbamoyl phosphate is provided primarily by the oxidative deamination of glutamate by mitochondrial glutamate dehydrogenase (see Figure 19.11). Ultimately, the nitrogen atom derived from this ammonia becomes one of the nitrogens of urea. CPS I

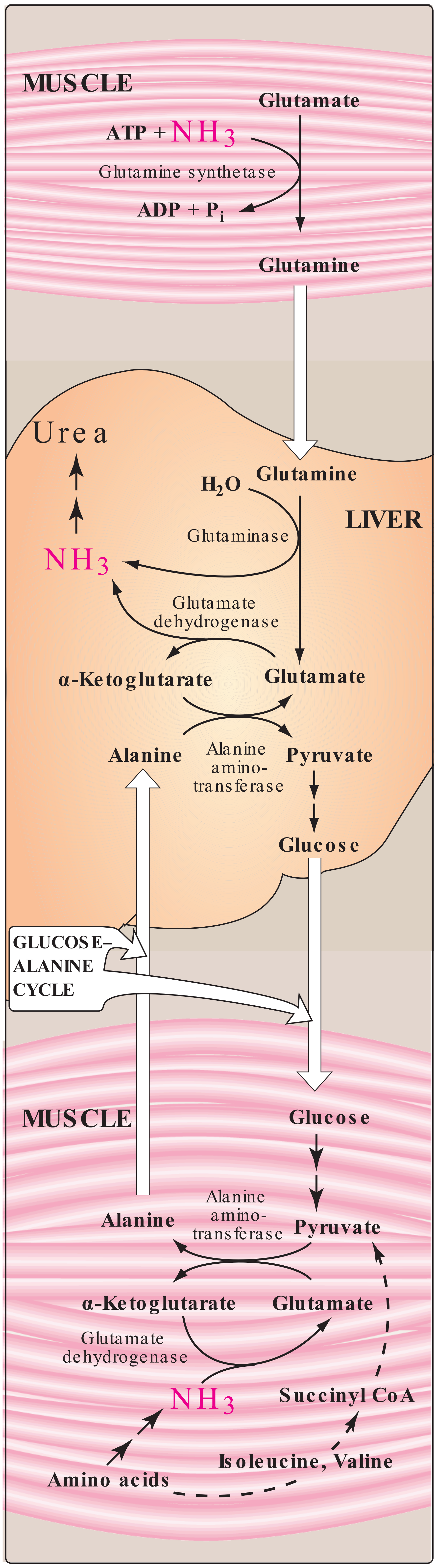
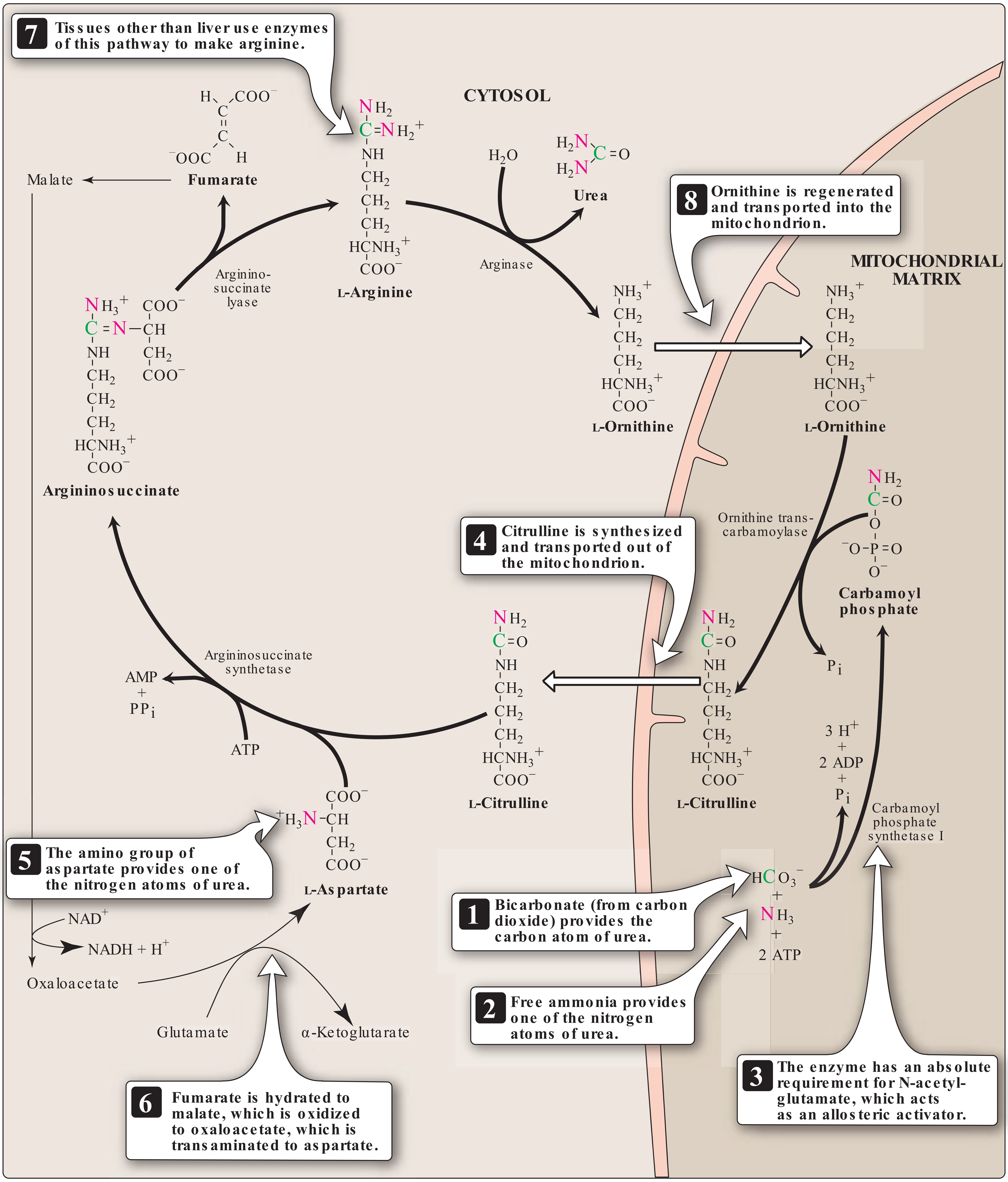

MUSCLE
Glutamate
ATP + NH3
Glutamine synthetase
ADP + Pi
Glutamine
Urea
H2O
Glutamine
LIVER
Glutaminase
NH3
Glutamate dehydrogenase
α-Ketoglutarate
Glutamate
Alanine
Alanine amino-transferase
Pyruvate
Glucose
GLUCOSE–ALANINE CYCLE
MUSCLE
Glucose
Alanine
Alanine amino-transferase
Pyruvate
α-Ketoglutarate
Glutamate
Glutamate dehydrogenase
NH3
Succinyl CoA
Isoleucine, Valine
Amino acids

Figure 19.13
Transport of ammonia (NH_3) from muscle to the liver. ADP = adenosine diphosphate; P_i = inorganic phosphate; CoA = coenzyme A.


CYTOSOL
MITOCHONDRIAL MATRIX
Fumarate
Malate
L-Arginine
Argininosuccinate lyase
H₂O
Urea
Arginase
L-Ornithine
L-Ornithine
Argininosuccinate
Ornithine transcarbamoylase
Carbamoyl phosphate
Pᵢ
Argininosuccinate synthetase
AMP + PPᵢ
ATP
L-Citrulline
L-Citrulline
3 H⁺ + 2 ADP + Pᵢ
Carbamoyl phosphate synthetase I
L-Aspartate
HCO₃⁻ + NH₃ + 2 ATP
NAD⁺
NADH + H⁺
Oxaloacetate
Glutamate
α-Ketoglutarate

7 Tissues other than liver use enzymes of this pathway to make arginine.

8 Ornithine is regenerated and transported into the mitochondrion.

4 Citrulline is synthesized and transported out of the mitochondrion.

5 The amino group of aspartate provides one of the nitrogen atoms of urea.

1 Bicarbonate (from carbon dioxide) provides the carbon atom of urea.

2 Free ammonia provides one of the nitrogen atoms of urea.

3 The enzyme has an absolute requirement for N-acetylglutamate, which acts as an allosteric activator.

6 Fumarate is hydrated to malate, which is oxidized to oxaloacetate, which is transaminated to aspartate.

Figure 19.14

Reactions of the urea cycle. [Note: An antiporter transports citrulline and ornithine across the inner mitochondrial membrane.] ADP = adenosine diphosphate; AMP = adenosine monophosphate; PP_i = pyrophosphate; P_i = inorganic phosphate; NAD(H) = nicotinamide adenine dinucleotide.

requires N-acetylglutamate as a positive allosteric activator (see Figure 19.14). [Note: Carbamoyl phosphate synthetase II participates in the biosynthesis of pyrimidines (see p. 302). It does not require N-acetylglutamate, uses glutamine as the nitrogen source, and occurs in the cytosol.]

2. **Formation of citrulline:** The carbamoyl portion of carbamoyl phosphate is transferred to ornithine by ornithine transcarbamoylase (OTC) as the high-energy phosphate is released as inorganic phosphate. The reaction product, citrulline, is transported to the cytosol. [Note: Ornithine and citrulline are basic amino acids that participate in the urea cycle, moving across the inner mitochondrial membrane via a cotransporter. They are not incorporated into cellular proteins because there are no codons for these amino acids (see p. 432).] Ornithine is regenerated with each turn of the urea cycle, much in the same way that oxaloacetate is regenerated by the reactions of the citric acid cycle (see p. 109).

3. **Synthesis of argininosuccinate:** Argininosuccinate synthetase combines citrulline with aspartate to form argininosuccinate. The α-amino group of aspartate provides the second nitrogen that is ultimately incorporated into urea. The formation of argininosuccinate is driven by the cleavage of ATP to adenosine monophosphate and pyrophosphate. This is the third and final molecule of ATP consumed in the formation of urea.

4. **Cleavage of argininosuccinate:** Argininosuccinate is cleaved by argininosuccinate lyase to yield arginine and fumarate. The arginine formed by this reaction serves as the immediate precursor of urea. Fumarate produced in the urea cycle is hydrated to malate, providing a link with several metabolic pathways. For example, the malate can be transported into the mitochondria via the malate–aspartate shuttle, reenter the tricarboxylic acid cycle, and get oxidized to oxaloacetate, which can be used for gluconeogenesis (see p. 120). [Note: Malate oxidation generates NADH and, subsequently, ATP.] Alternatively, the oxaloacetate can be converted to aspartate via transamination (see Figure 19.8) and can enter the urea cycle (see Figure 19.14).

5. **Cleavage of arginine to ornithine and urea:** Arginase hydrolyzes arginine to ornithine and urea and is virtually exclusive to the liver. Therefore, only the liver can cleave arginine, thereby synthesizing urea, whereas other tissues, such as the kidney, can synthesize arginine by these reactions.

6. **Fate of urea:** Urea diffuses from the liver, and is transported in the blood to the kidneys, where it is filtered and excreted in the urine (see Figure 19.19). A portion of the urea diffuses from the blood into the intestine and is cleaved to CO_2 and NH_3 by bacterial urease. This ammonia is partly lost in the feces and is partly reabsorbed into the blood. In patients with kidney failure, plasma urea levels are elevated, promoting a greater transfer of urea from blood into the gut. The intestinal action of urease on this urea becomes a clinically important source of ammonia, contributing to the hyperammonemia often seen in these patients. Oral administration of antibiotics reduces the number of intestinal bacteria responsible for this NH_3 production.

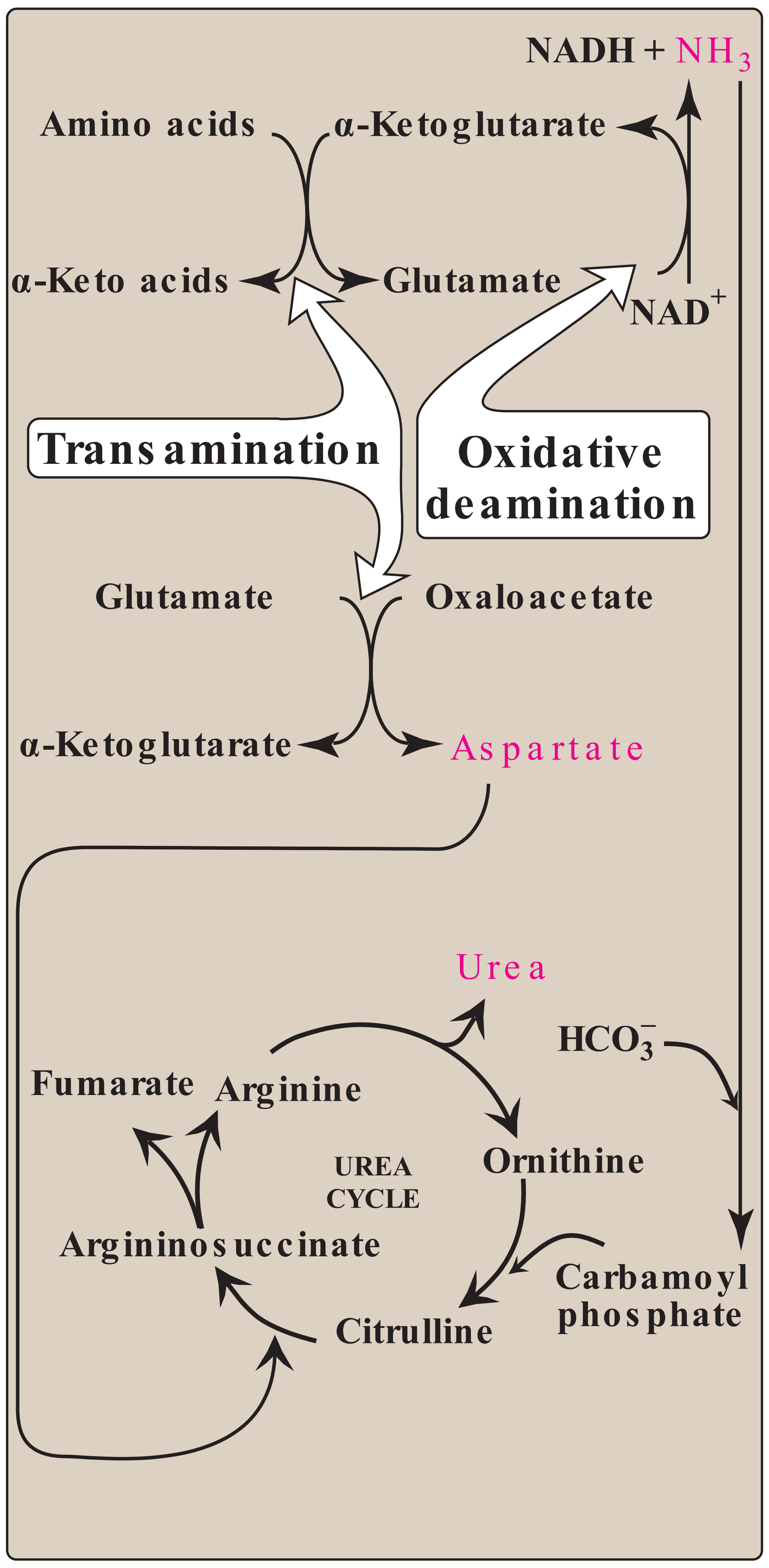


Figure 19.15
Flow of nitrogen from amino acids to urea. Amino groups for urea synthesis are collected in the form of ammonia and aspartate. NAD(H) = nicotinamide adenine dinucleotide.

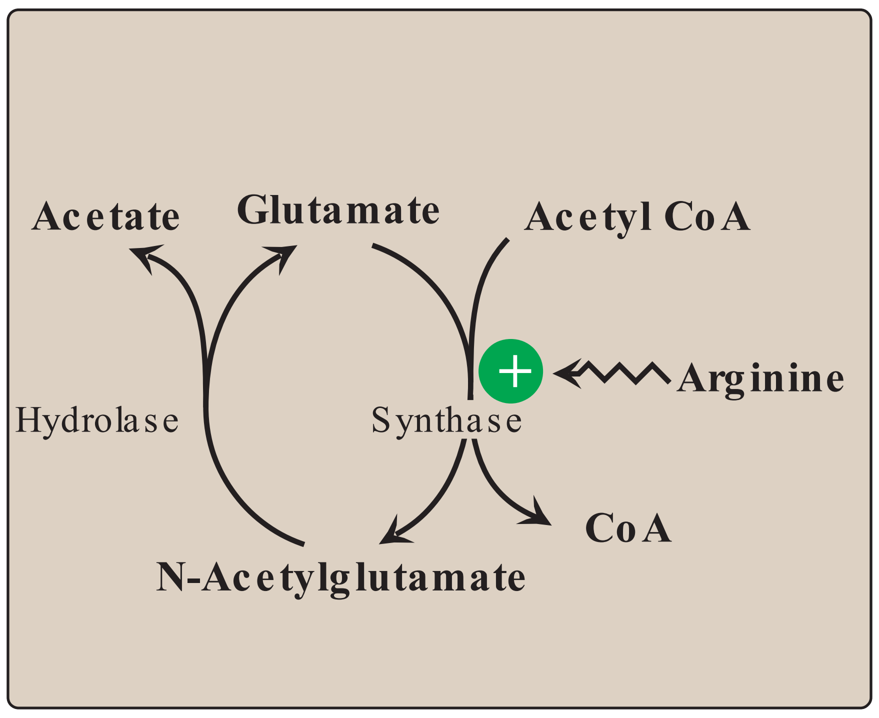


Figure 19.16
Formation and degradation of N-acetylglutamate, an allosteric activator of carbamoyl phosphate synthetase I. CoA = coenzyme A.

B. Overall stoichiometry of the urea cycle

$$\text{Aspartate} + NH_3 + HCO_3^- + 3\ \text{ATP} + H_2O \longrightarrow$$

$$\text{urea} + \text{fumarate} + 2\ \text{ADP} + \text{AMP} + 2\ P_i + PP_i$$

Because four high-energy phosphate bonds are consumed in the synthesis of each molecule of urea, the synthesis of urea is irreversible, with a large, negative ΔG (see p. 70). One nitrogen of the urea molecule is supplied by free NH_3, and the other nitrogen by aspartate. Glutamate is the immediate precursor of both ammonia (through oxidative deamination by glutamate dehydrogenase) and aspartate nitrogen (through transamination of oxaloacetate by AST). In effect, both nitrogen atoms of urea arise from glutamate, which, in turn, gathers nitrogen from other amino acids (Figure 19.15).

C. Regulation of the urea cycle

N-Acetylglutamate (NAG) is an essential activator for CPS I, the rate-limiting step in the urea cycle. It increases the affinity of CPS I for ATP. NAG is synthesized from acetyl CoA and glutamate by N-acetylglutamate synthase (Figure 19.16) in a reaction for which arginine is an activator. The cycle is also regulated by substrate availability (short-term regulation) and enzyme induction (long term).

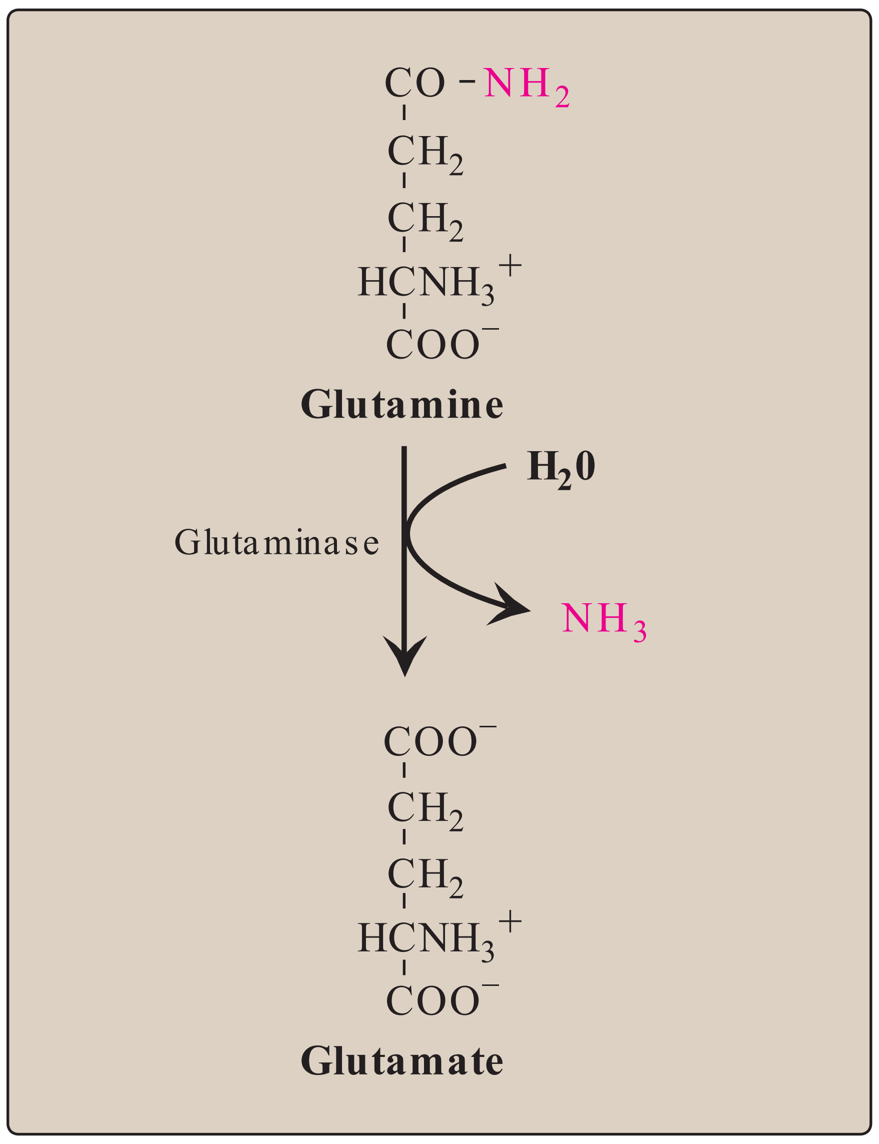


Figure 19.17
Hydrolysis of glutamine to form ammonia (NH_3).

VII. METABOLISM OF AMMONIA

Ammonia is produced by all tissues during the metabolism of a variety of compounds, and it is disposed of primarily by formation of urea in the liver. However, the level of ammonia in the blood must be kept very low, because even slightly elevated concentrations (hyperammonemia) are toxic to the central nervous system (CNS). Therefore, there must be a metabolic mechanism by which nitrogen is moved from peripheral tissues to the liver for ultimate disposal as urea, at the same time maintaining low levels of circulating ammonia.

A. Sources of ammonia

Amino acids are quantitatively the most important source of ammonia because most Western diets are high in protein and provide excess amino acids, which travel to the liver and undergo transdeamination (that is, the linking of aminotransferase and glutamate dehydrogenase reactions), producing ammonia. [Note: Liver catabolizes straight-chain amino acids, primarily.] However, substantial amounts of ammonia can be obtained from other sources.

1. **From glutamine:** An important source of plasma glutamine is from the catabolism of branched-chain amino acids in skeletal muscle. This glutamine is taken up by cells of the intestine, the liver, and the kidney.The liver and kidneys generate ammonia from glutamine by the actions of glutaminase (Figure 19.17) and glutamate dehydrogenase. In the kidneys, most of this ammonia is excreted into the urine as NH_4^+, which provides an important mechanism for maintaining the body's acid–base balance through the excretion of protons. In the liver, the ammonia is detoxified to urea and excreted. [Note: α-Ketoglutarate, the second product of glutamate dehydrogenase, is a glucogenic precursor in liver and kidney.]

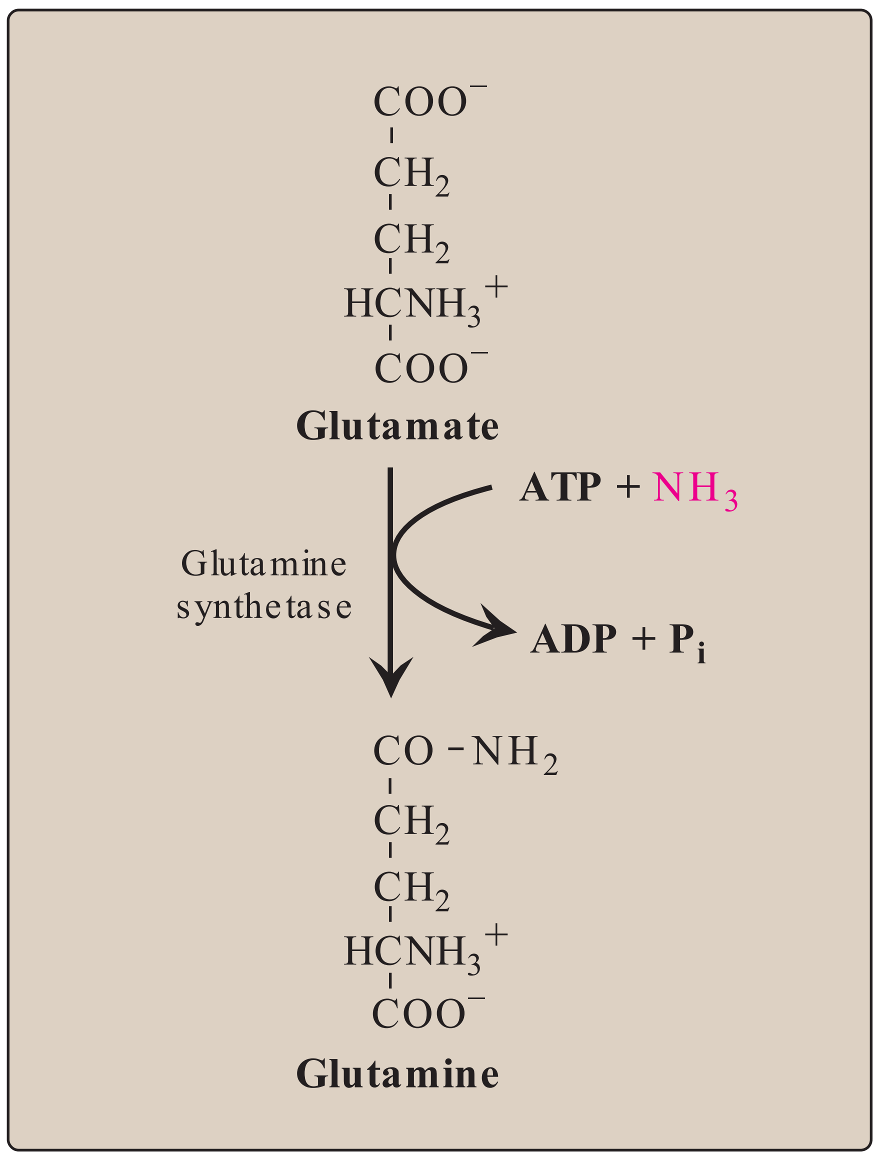


Figure 19.18
Synthesis of glutamine.
ADP = adenosine diphosphate;
P_i = inorganic phosphate.

Ammonia is also generated by intestinal glutaminase. The intestinal mucosal cells obtain glutamine either from the blood or from digestion of dietary protein. [Note: Intestinal glutamine metabolism also produces alanine, which is used by the liver for gluconeogenesis, and citrulline, which is used by the kidneys to synthesize arginine.]

2. **From bacterial action in the intestine:** Ammonia is formed from urea by the action of bacterial urease in the lumen of the intestine. This ammonia is absorbed from the intestine by way of the portal vein, and virtually all is removed by the liver via conversion to urea.
3. **From amines:** Amines obtained from the diet and monoamines that serve as hormones or neurotransmitters give rise to ammonia by the action of monoamine oxidase (see p. 286).
4. **From purines and pyrimidines:** In the catabolism of purines and pyrimidines, amino groups attached to the ring atoms are released as ammonia (see Figure 22.15 and p. 304).

B. Transport of ammonia in the circulation

Although ammonia is constantly produced in the tissues, it is present at very low levels in blood. This is due both to the rapid removal of blood ammonia by the liver and to the fact that several tissues, particularly muscle, release amino acid nitrogen in the form of glutamine or alanine, rather than as free ammonia (see Figure 19.13).

1. **Urea:** Formation of urea in the liver is quantitatively the most important disposal route for ammonia. Urea travels in the blood from the liver to the kidneys, where it passes into the glomerular filtrate.
2. **Glutamine:** This amide of glutamate provides a nontoxic storage and transport form of ammonia (Figure 19.18). The ATP-requiring formation of glutamine from glutamate and ammonia by glutamine synthetase occurs primarily in skeletal muscle and liver but is also important in the CNS, where it is the major mechanism for the removal of ammonia in the brain. Glutamine is found in plasma at concentrations higher than other amino acids, a finding consistent with its transport function. [Note: The liver keeps blood ammonia levels low through glutaminase and the urea cycle in periportal (close to inflow of blood) hepatocytes and via glutamine synthetase as an ammonia "scavenger" in the perivenous hepatocytes.] The metabolism of ammonia is summarized in Figure 19.19.

C. Hyperammonemia

The capacity of the hepatic urea cycle exceeds the normal rates of ammonia generation, and the levels of serum ammonia are normally low (5–35 µmol/l). However, when liver function is compromised, due either to genetic defects of the urea cycle or liver disease, blood levels can rise above 1,000 µmol/l. Such hyperammonemia is a medical emergency, because ammonia has a direct neurotoxic effect on the CNS. For example, elevated concentrations of ammonia in the blood cause the symptoms of ammonia intoxication, which include tremors, slurring of speech, somnolence (drowsiness), vomiting, cerebral edema, and blurring of vision. At high concentrations, ammonia can cause coma and death. There are two major types of hyperammonemia.

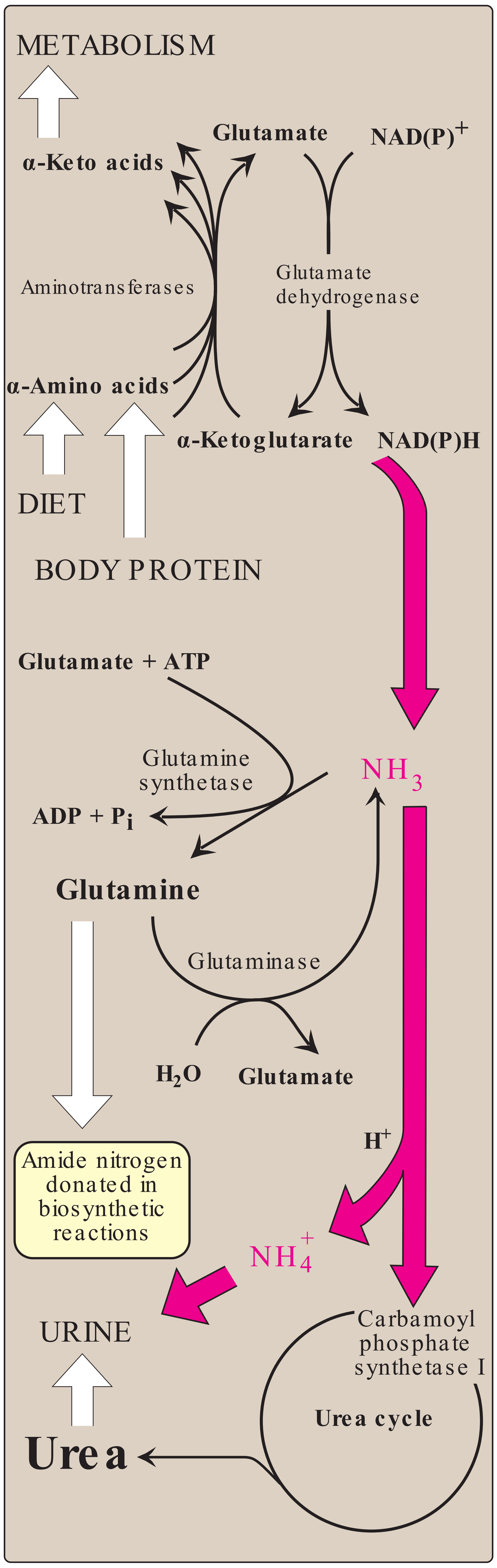
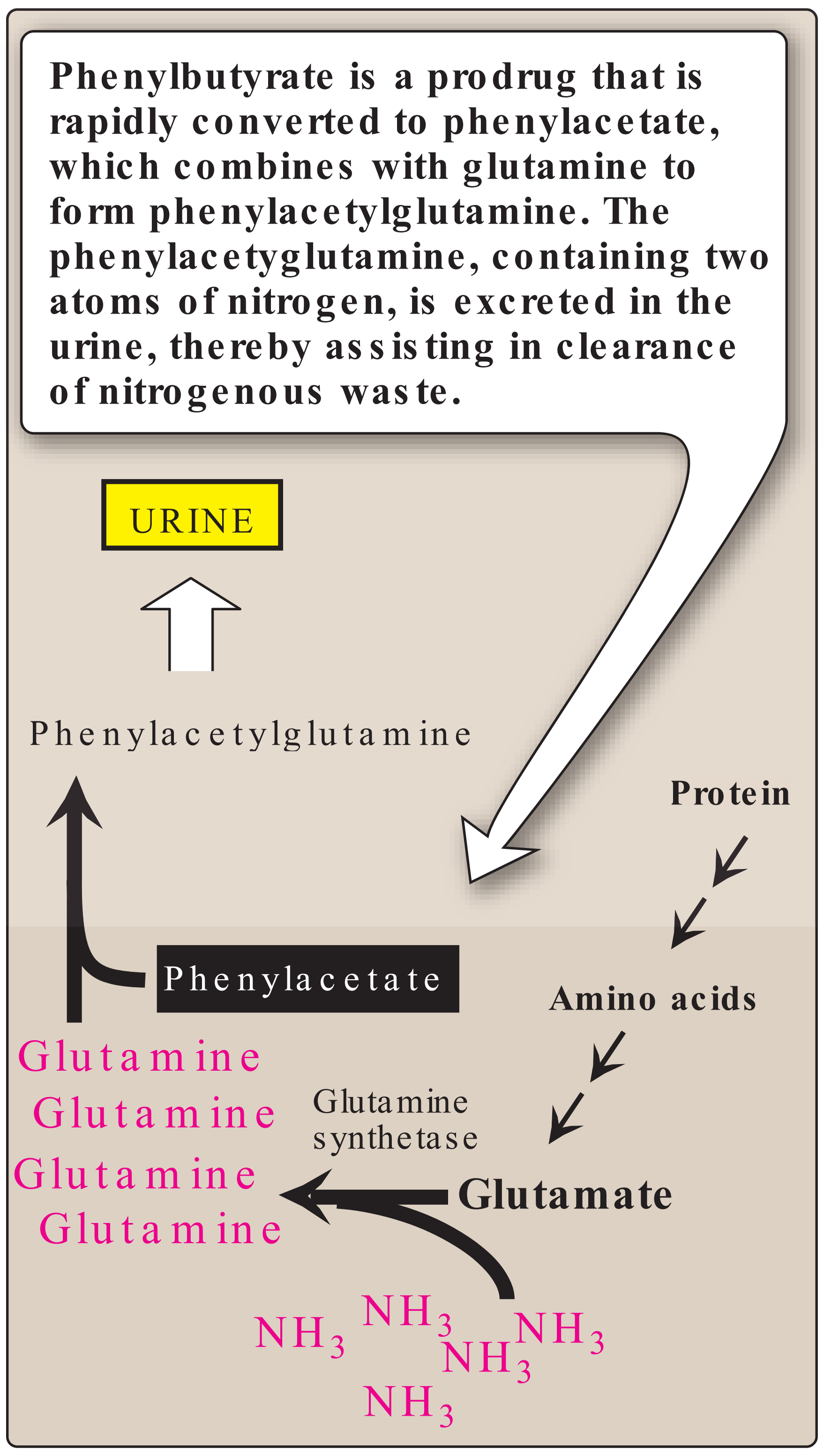


Figure 19.19

Metabolism of ammonia (NH_3). [Note: Glutamate dehydrogenase is one of several sources of NH_3.] Urea content in the urine is reported as urinary urea nitrogen, or UUN. Urea in blood is reported as BUN (blood urea nitrogen). The enzymes glutamate dehydrogenase, glutamine synthetase, and carbamoyl phosphate synthetase I fix NH_3 into organic molecules.



Phenylbutyrate is a prodrug that is rapidly converted to phenylacetate, which combines with glutamine to form phenylacetylglutamine. The phenylacetyglutamine, containing two atoms of nitrogen, is excreted in the urine, thereby assisting in clearance of nitrogenous waste.

Figure 19.20
Treatment of patients with urea cycle defects by administration of phenylbutyrate to aid in excretion of ammonia (NH_3).

1. **Acquired hyperammonemia:** Liver disease is a common cause of hyperammonemia in adults and may be due, for example, to viral hepatitis or to hepatotoxins such as alcohol. Cirrhosis of the liver may result in formation of collateral circulation around the liver. As a result, portal blood is shunted directly into the systemic circulation and does not have access to the liver. Therefore, the conversion of ammonia to urea is severely impaired, leading to elevated levels of ammonia.

2. **Congenital hyperammonemia:** Genetic deficiencies of each of the five enzymes of the urea cycle have been described, with an overall incidence estimated to be 1:25,000 live births. X-linked ornithine transcarbamoylase deficiency is the most common of these disorders, predominantly affecting males, although female carriers may become symptomatic. All of the other urea cycle disorders follow an autosomal-recessive inheritance pattern. In each case, the failure to synthesize urea leads to hyperammonemia during the first weeks following birth. [Note: The hyperammonemia seen with arginase deficiency is less severe because arginine contains two waste nitrogens and can be excreted in the urine.] Historically, urea cycle defects had high morbidity (neurologic manifestations) and mortality. Treatment included restriction of dietary protein in the presence of sufficient calories to prevent catabolism. Administration of compounds that bind covalently to amino acids, producing nitrogen-containing molecules that are excreted in the urine, has improved survival. For example, phenylbutyrate given orally is converted to phenylacetate. This condenses with glutamine to form phenylacetylglutamine, which is excreted (Figure 19.20).

VIII. CHAPTER SUMMARY

Nitrogen enters the body in a variety of compounds present in food, the most important being amino acids contained in **dietary protein**. **Nitrogen leaves** the body as **urea**, **ammonia**, and other products derived from amino acid metabolism (Figure 19.21). Free amino acids in the body are produced by hydrolysis of dietary protein by proteases activated from their zymogen form in the stomach and intestine, degradation of tissue proteins, and de novo synthesis. This **amino acid pool** is consumed in the synthesis of body protein, metabolized for energy, or its members used as precursors for other nitrogen-containing compounds. Free amino acids from digestion are taken up by intestinal cells via sodium-linked secondary active transport. Note that body protein is simultaneously degraded and resynthesized, a process known as **protein turnover**. The concentration of a cellular protein may be determined by regulation of its synthesis or degradation. The adenosine triphosphate (ATP)-dependent, cytosolic, selective **ubiquitin–proteasome** and ATP-independent, nonselective **lysosomal** acid hydrolases are the two major enzyme systems that are responsible for **degrading proteins**. Nitrogen cannot be stored, and amino acids in excess of the biosynthetic needs of the cell are quickly degraded. The first phase of **catabolism** involves the transfer of the α-amino groups through transamination by **pyridoxal phosphate**–dependent aminotransferases (transaminases), followed by **oxidative deamination of glutamate** by glutamate dehydrogenase, forming **ammonia** and the corresponding **α-keto acids**. A portion of the **free ammonia** is excreted in the **urine**, some of which is used in converting glutamate to glutamine for safe transport, but most is used in the hepatic synthesis of **urea**, which is quantitatively the most important route for disposing of nitrogen from the body. The two major causes of **hyperammonemia** (with its neurologic effects) are liver disease and inherited deficiencies of urea cycle enzymes such as X-linked ornithine transcarbamolyase.

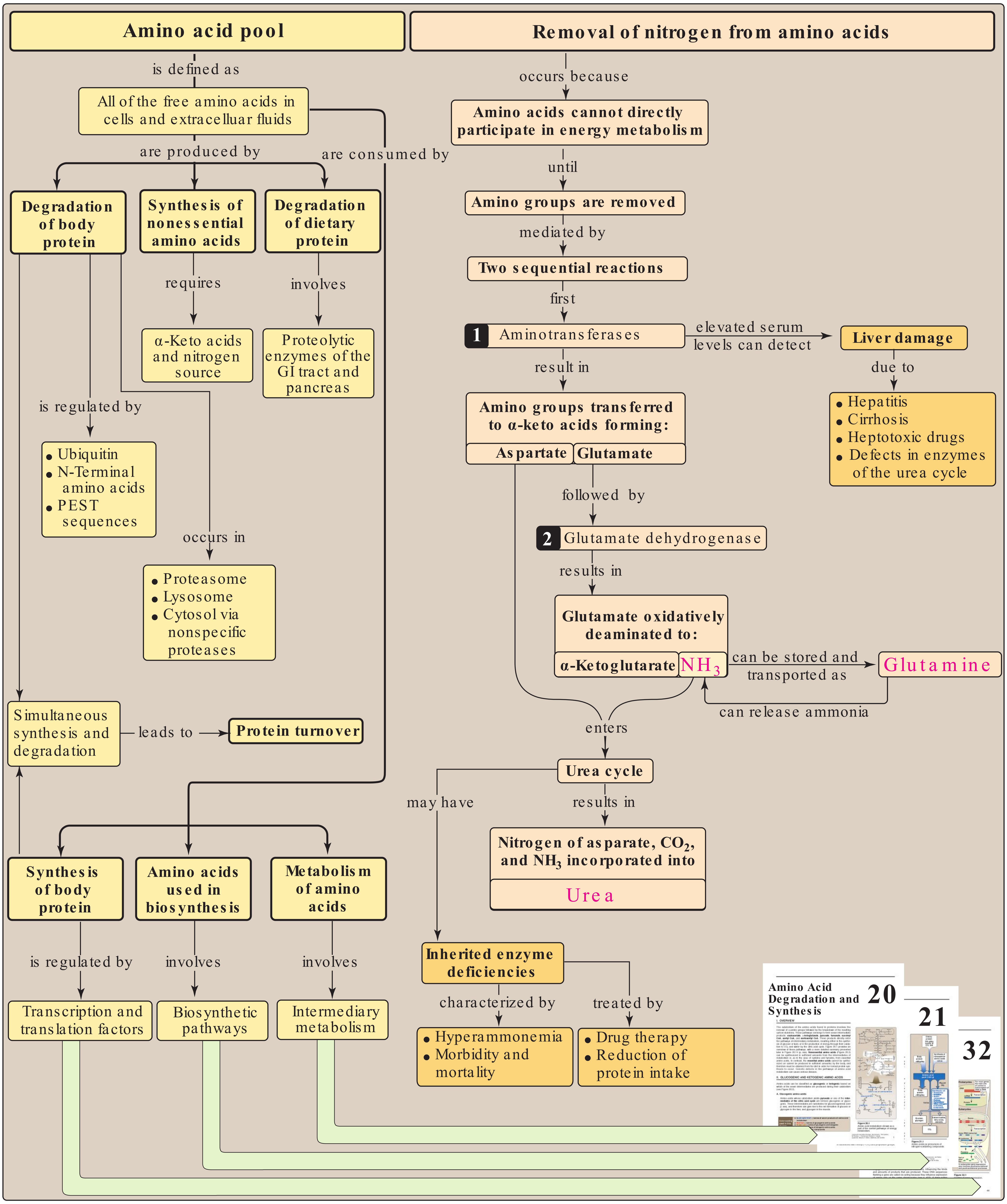

Amino acid pool
is defined as
All of the free amino acids in cells and extracellular fluids
are produced by
are consumed by
Degradation of body protein
Synthesis of nonessential amino acids
Degradation of dietary protein
requires
involves
α-Keto acids and nitrogen source
Proteolytic enzymes of the GI tract and pancreas
is regulated by
• Ubiquitin
• N-Terminal amino acids
• PEST sequences
occurs in
• Proteasome
• Lysosome
• Cytosol via nonspecific proteases
Simultaneous synthesis and degradation
leads to
Protein turnover
Synthesis of body protein
Amino acids used in biosynthesis
Metabolism of amino acids
is regulated by
involves
involves
Transcription and translation factors
Biosynthetic pathways
Intermediary metabolism
Removal of nitrogen from amino acids
occurs because
Amino acids cannot directly participate in energy metabolism
until
Amino groups are removed
mediated by
Two sequential reactions
first
1 Aminotransferases
elevated serum levels can detect
Liver damage
due to
• Hepatitis
• Cirrhosis
• Heptotoxic drugs
• Defects in enzymes of the urea cycle
result in
Amino groups transferred to α-keto acids forming:
Aspartate
Glutamate
followed by
2 Glutamate dehydrogenase
results in
Glutamate oxidatively deaminated to:
α-Ketoglutarate
NH_3
can be stored and transported as
Glutamine
can release ammonia
enters
Urea cycle
may have
results in
Nitrogen of asparate, CO_2, and NH_3 incorporated into
Urea
Inherited enzyme deficiencies
characterized by
treated by
• Hyperammonemia
• Morbidity and mortality
• Drug therapy
• Reduction of protein intake
Amino Acid Degradation and Synthesis
20
21
32

Figure 19.21
Key concept map for nitrogen metabolism. GI = gastrointestinal; PEST = proline, glutamate, serine, threonine; NH_3 = ammonia.

Amino Acid Degradation and Synthesis

20

I. OVERVIEW

The catabolism of the amino acids involves the removal of α-amino groups, followed by the degradation of the resulting carbon skeletons. These pathways converge to form seven intermediate products: oxaloacetate, pyruvate, α-ketoglutarate, fumarate, succinyl coenzyme A (CoA), acetyl CoA, and acetoacetate. These products directly enter the pathways of intermediary metabolism, resulting either in the synthesis of glucose or lipid or in the production of energy through their oxidation to CO_2 by the tricarboxylic acid (TCA) cycle. Figure 20.1 provides an overview of these pathways, with a more detailed summary presented in Figure 20.14 (see p. 269). Nonessential amino acids (Figure 20.2) can be synthesized in sufficient amounts from the intermediates of metabolism or, as in the case of cysteine and tyrosine, from essential amino acids. In contrast, the essential amino acids cannot be synthesized (or produced in sufficient amounts) by the body and, therefore, must be obtained from the diet in order for normal protein synthesis to occur. Genetic defects in the pathways of amino acid metabolism can cause serious disease.

II. GLUCOGENIC AND KETOGENIC AMINO ACIDS

Amino acids can be classified as glucogenic, ketogenic, or both, based on which of the seven intermediates are produced during their catabolism (see Figure 20.2).

A. Glucogenic amino acids

Amino acids whose catabolism yields pyruvate or one of the intermediates of the TCA cycle are termed glucogenic. These intermediates are substrates for gluconeogenesis (see p. 117) and, therefore, can give rise to the net synthesis of glucose in the liver and kidney.

Color-coding used in this chapter:

- BLUE CAPS TEXT = **names of seven products of amino acid metabolism**
- Red text = **names of glucogenic amino acids**
- Brown text = **names of glucogenic and ketogenic amino acids**
- Green text = **names of ketogenic amino acids**
- Cyan text = **one-carbon compounds**

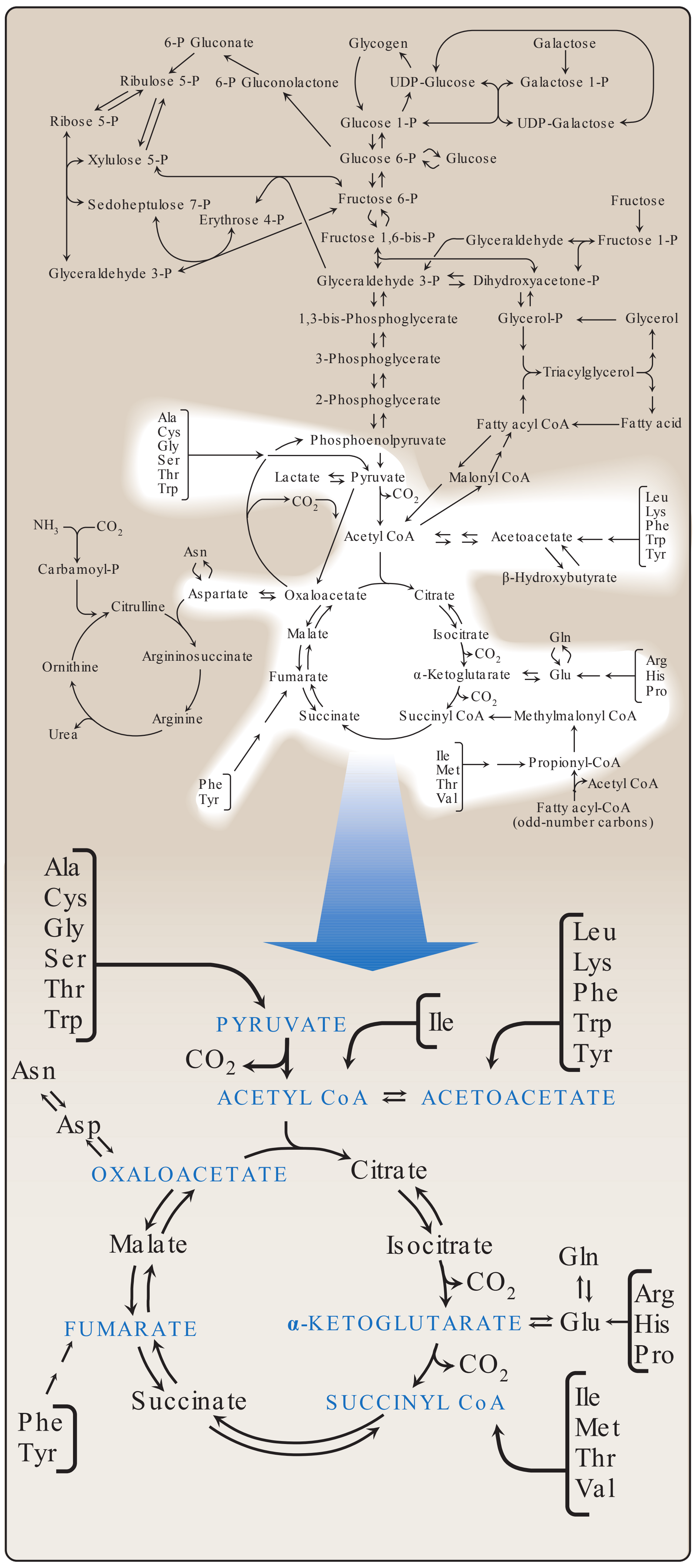
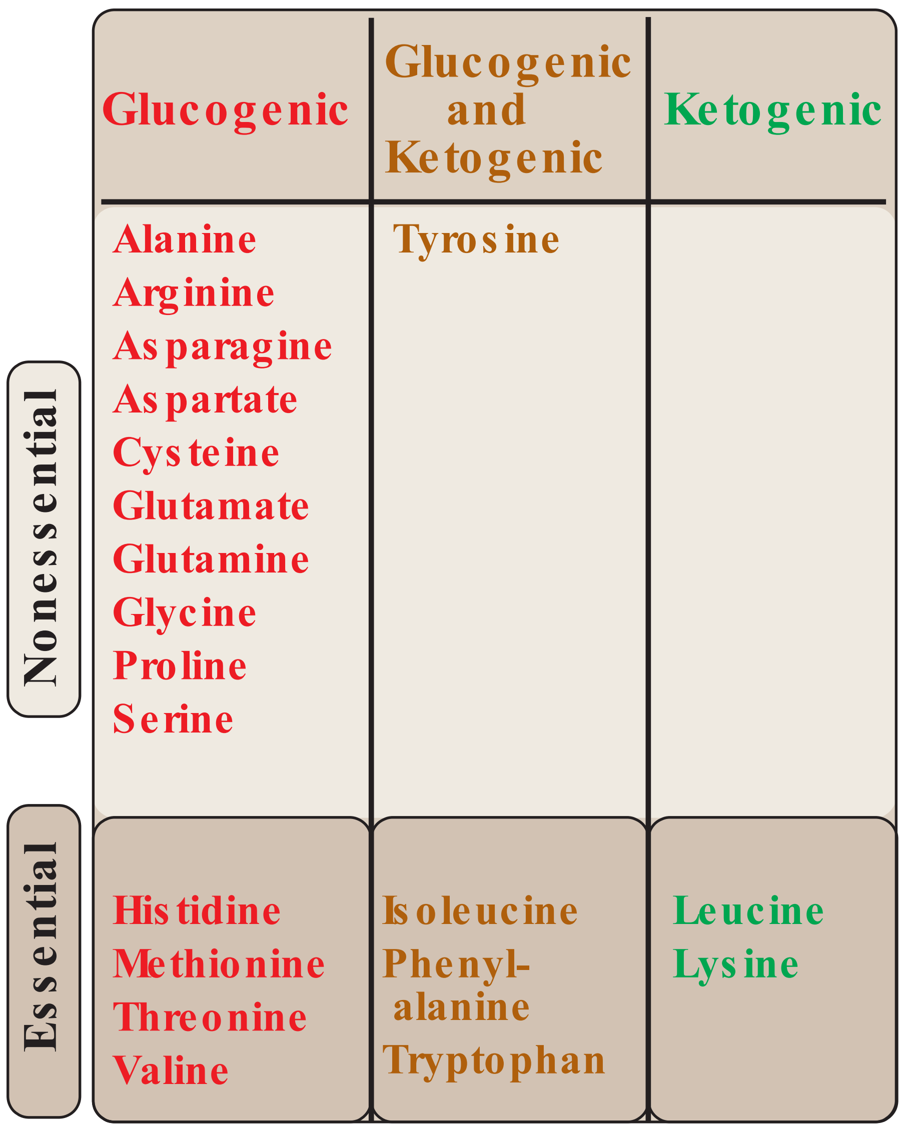

6-P Gluconate
Glycogen
Galactose
Ribulose 5-P
6-P Gluconolactone
UDP-Glucose
Galactose 1-P
Ribose 5-P
Glucose 1-P
UDP-Galactose
Xylulose 5-P
Glucose 6-P
Glucose
Sedoheptulose 7-P
Erythrose 4-P
Fructose 6-P
Fructose
Fructose 1,6-bis-P
Glyceraldehyde
Fructose 1-P
Glyceraldehyde 3-P
Glyceraldehyde 3-P
Dihydroxyacetone-P
1,3-bis-Phosphoglycerate
Glycerol-P
Glycerol
3-Phosphoglycerate
Triacylglycerol
2-Phosphoglycerate
Fatty acyl CoA
Fatty acid
Ala
Cys
Gly
Ser
Thr
Trp
Phosphoenolpyruvate
Lactate
Pyruvate
Malonyl CoA
CO2
CO2
Leu
Lys
Phe
Trp
Tyr
NH3
CO2
Acetyl CoA
Acetoacetate
Asn
Carbamoyl-P
β-Hydroxybutyrate
Citrulline
Aspartate
Oxaloacetate
Citrate
Malate
Isocitrate
Gln
Ornithine
Argininosuccinate
CO2
Arg
His
Pro
Fumarate
α-Ketoglutarate
Glu
CO2
Urea
Arginine
Succinate
Succinyl CoA
Methylmalonyl CoA
Ile
Met
Thr
Val
Propionyl-CoA
Acetyl CoA
Phe
Tyr
Fatty acyl-CoA (odd-number carbons)
Ala
Cys
Gly
Ser
Thr
Trp
Leu
Lys
Phe
Trp
Tyr
PYRUVATE
Ile
CO2
Asn
ACETYL CoA
ACETOACETATE
Asp
OXALOACETATE
Citrate
Malate
Isocitrate
Gln
CO2
Arg
His
Pro
FUMARATE
α-KETOGLUTARATE
Glu
CO2
Phe
Tyr
Succinate
SUCCINYL CoA
Ile
Met
Thr
Val

Figure 20.1
Amino acid metabolism shown as a part of the essential pathways of energy metabolism. (See Figure 8.2, p. 92, for a more detailed view of these processes.) CoA = coenzyme A.



	Glucogenic	Glucogenic and Ketogenic	Ketogenic
Nonessential	Alanine Arginine Asparagine Aspartate Cysteine Glutamate Glutamine Glycine Proline Serine	Tyrosine	
Essential	Histidine Methionine Threonine Valine	Isoleucine Phenylalanine Tryptophan	Leucine Lysine

Figure 20.2
Classification of amino acids. [Note: Some amino acids can become conditionally essential. For example, supplementation with glutamine and arginine has been shown to improve outcomes in patients with trauma, postoperative infections, and immunosuppression.]

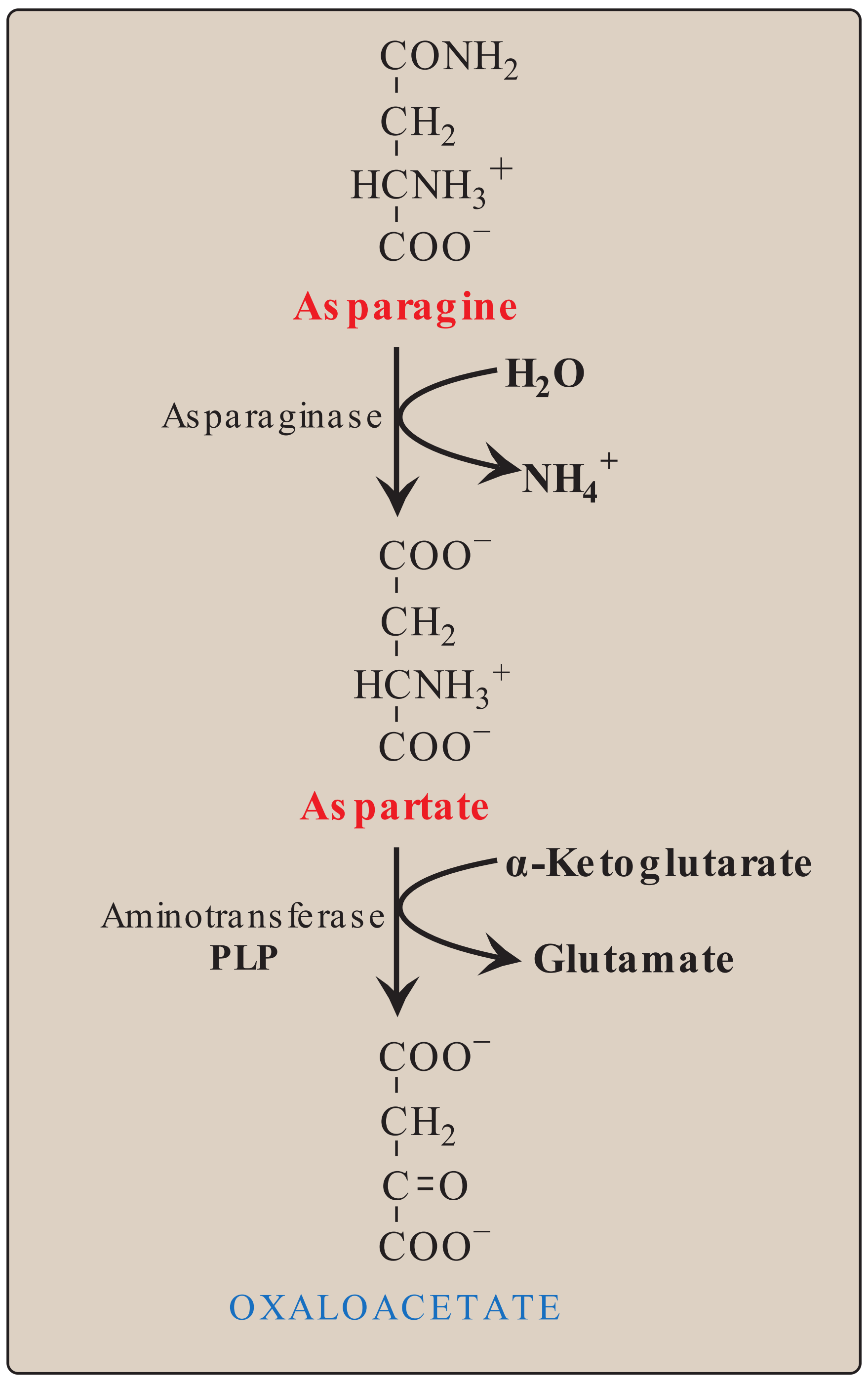


Figure 20.3
Metabolism of asparagine and aspartate. [Note: Recall that carbons from aspartate give rise to fumarate in the urea cycle (see p. 254).] PLP = pyridoxal phosphate.

B. Ketogenic amino acids

Amino acids whose catabolism yields either acetoacetate or one of its precursors (acetyl CoA or acetoacetyl CoA) are termed ketogenic (see Figure 20.2). Acetoacetate is one of the ketone bodies, which also include 3-hydroxybutyrate and acetone (see p. 195). Leucine and lysine are the only exclusively ketogenic amino acids found in proteins. Their carbon skeletons are not substrates for gluconeogenesis and, therefore, cannot give rise to the net synthesis of glucose.

III. CATABOLISM OF THE CARBON SKELETONS OF AMINO ACIDS

The pathways by which amino acids are catabolized are conveniently organized according to which one (or more) of the seven intermediates listed above is produced from a particular amino acid.

A. Amino acids that form oxaloacetate

Asparagine is hydrolyzed by asparaginase, liberating ammonium (NH_4^+) and aspartate (Figure 20.3). Aspartate loses its amino group by transamination to form oxaloacetate (see Figure 20.3). [Note: Some rapidly dividing leukemic cells are unable to synthesize sufficient asparagine to support their growth. This makes asparagine an essential amino acid for these cells, which, therefore, require asparagine from the blood. Asparaginase, which hydrolyzes asparagine to aspartate, can be administered systemically to treat leukemic patients. Asparaginase lowers the level of asparagine in the plasma, thereby depriving cancer cells of a required nutrient.]

B. Amino acids that form α-ketoglutarate via glutamate

1. **Glutamine:** This amino acid is hydrolyzed to glutamate and ammonium by the enzyme glutaminase (see p. 256). Glutamate is converted to α-ketoglutarate by transamination or through oxidative deamination by glutamate dehydrogenase (see p. 252).

2. **Proline:** This amino acid is oxidized to glutamate. Glutamate is transaminated or oxidatively deaminated to form α-ketoglutarate.

3. **Arginine:** This amino acid is hydrolyzed by arginase to produce ornithine (and urea). [Note: This reaction occurs primarily in the liver as part of the urea cycle (see p. 255).] Ornithine is subsequently converted to α-ketoglutarate, with glutamate semialdehyde as an intermediate.

4. **Histidine:** This amino acid is oxidatively deaminated by histidase to urocanic acid, which subsequently forms N-formiminoglutamate ([FIGlu] Figure 20.4). FIGlu donates its formimino group to tetrahydrofolate (THF), leaving glutamate, which is degraded as described above. [Note: Individuals deficient in folic acid excrete increased amounts of FIGlu in the urine, particularly after ingestion of a large dose of histidine. The FIGlu excretion test has been used in diagnosing a deficiency of folic acid.] (See p. 267 for a discussion of folic acid, THF, and one-carbon metabolism.)

C. Amino acids that form pyruvate

1. **Alanine:** This amino acid loses its amino group by transamination to form pyruvate (Figure 20.5). [Note: Alanine is the major glucogenic amino acid.]

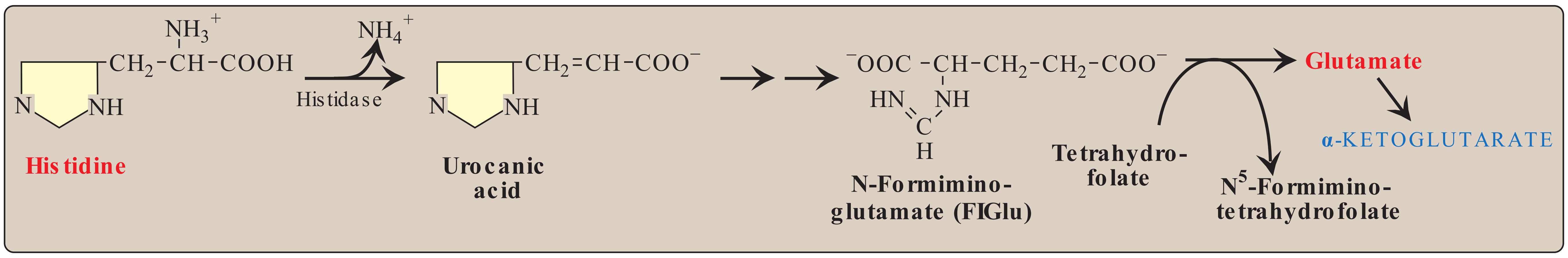

NH3+
CH2-CH-COOH
N
NH
Histidine
NH4+
Histidase
CH2=CH-COO−
N
NH
Urocanic acid
−OOC-CH-CH2-CH2-COO−
HN NH
C
H
N-Formimino-glutamate (FIGlu)
Tetrahydro-folate
Glutamate
α-KETOGLUTARATE
N5-Formimino-tetrahydrofolate

Figure 20.4
Degradation of histidine.

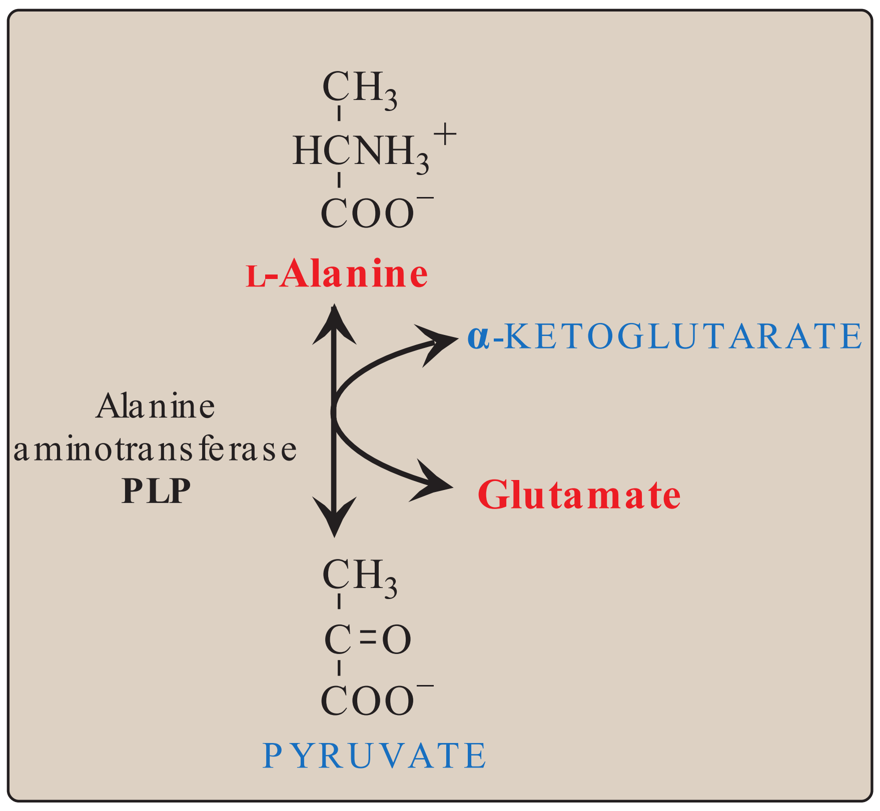

CH3
HCNH3+
COO−
L-Alanine
α-KETOGLUTARATE
Alanine aminotransferase
PLP
Glutamate
CH3
C=O
COO−
PYRUVATE

Figure 20.5
Transamination of alanine to form pyruvate. PLP = pyridoxal phosphate.

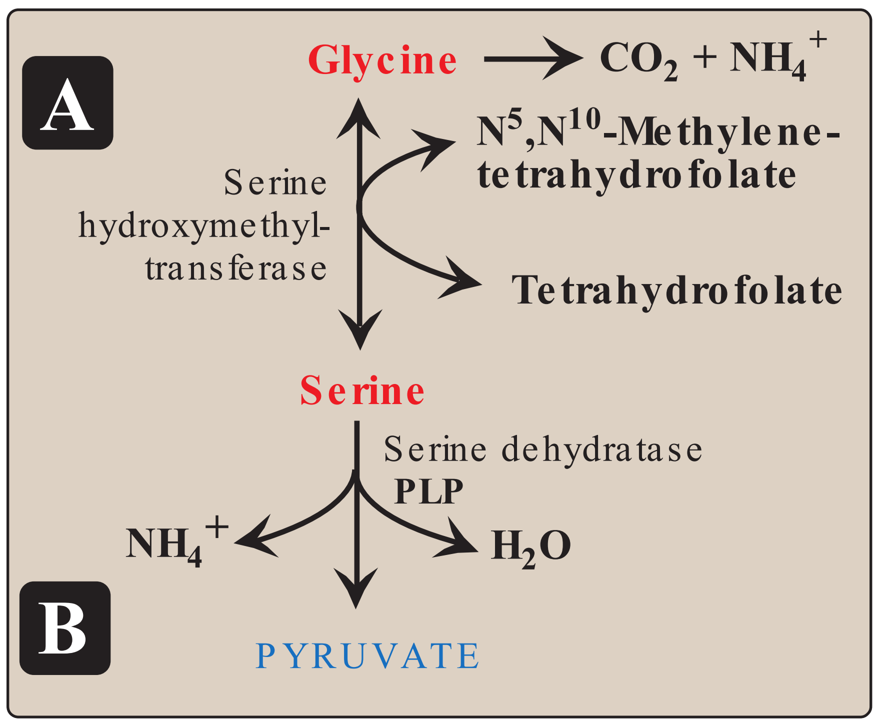

A
Glycine
CO2 + NH4+
N5,N10-Methylene-tetrahydrofolate
Serine hydroxymethyl-transferase
Tetrahydrofolate
Serine
Serine dehydratase
PLP
NH4+
H2O
B
PYRUVATE

Figure 20.6
A. Interconversion of serine and glycine and oxidation of glycine.
B. Dehydration of serine to form pyruvate. PLP = pyridoxal phosphate.

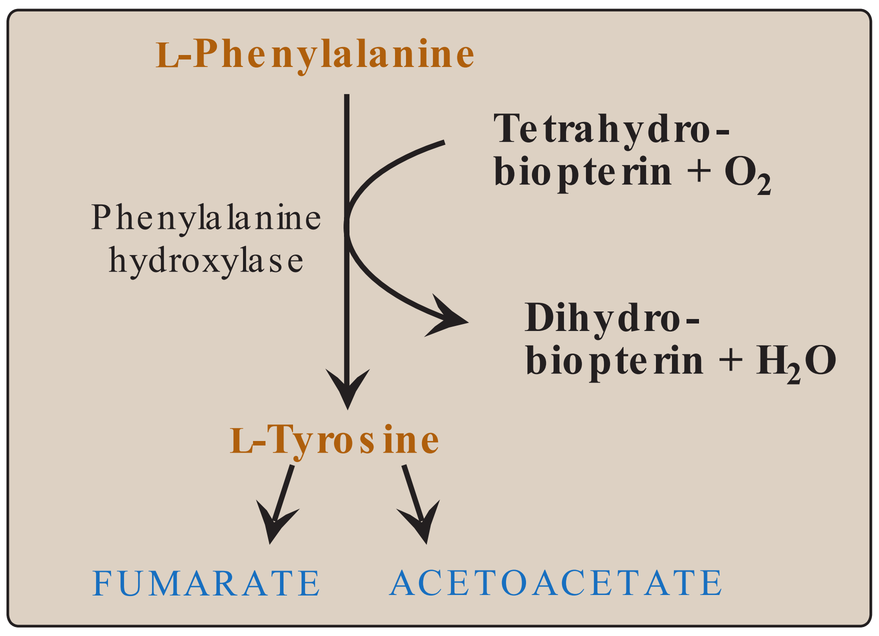

L-Phenylalanine
Tetrahydro-biopterin + O2
Phenylalanine hydroxylase
Dihydro-biopterin + H2O
L-Tyrosine
FUMARATE
ACETOACETATE

Figure 20.7
Degradation of phenylalanine.

2. **Serine:** This amino acid can be converted to glycine and N^5,N^{10}-methylenetetrahydrofolate (Figure 20.6A). Serine can also be converted to pyruvate by serine dehydratase (Figure 20.6B).

3. **Glycine:** This amino acid can be converted to serine by the reversible addition of a methylene group from N^5,N^{10}-methylenetetrahydrofolic acid (see Figure 20.6A) or oxidized to CO_2 and NH_4^+. [Note: Glycine can be deaminated to glyoxylate, which can be oxidized to oxalate or transaminated to glycine. Deficiency of the transaminase in liver peroxisomes causes overproduction of oxalate, the formation of oxalate stones, and kidney damage (primary oxaluria type 1).]

4. **Cysteine:** This amino acid undergoes desulfuration to yield pyruvate. [Note: The sulfate released can be used to synthesize 3'-phosphoadenosine-5'-phosphosulfate (PAPS), an activated sulfur donor to a variety of acceptors.] Cysteine can be oxidized to its disulfide derivative, cystine.

5. **Threonine:** This amino acid is converted to pyruvate in most organisms but is a minor pathway (at best) in humans.

D. Amino acids that form fumarate

1. **Phenylalanine and tyrosine:** Hydroxylation of phenylalanine produces tyrosine (Figure 20.7). This reaction, catalyzed by tetrahydrobiopterin-requiring phenylalanine hydroxylase, initiates the catabolism of phenylalanine. Thus, the metabolism of phenylalanine and tyrosine merge, leading ultimately to the formation of fumarate and acetoacetate. Phenylalanine and tyrosine are, therefore, both glucogenic and ketogenic.

2. **Inherited deficiencies:** Inherited deficiencies in the enzymes of phenylalanine and tyrosine metabolism lead to the diseases phenylketonuria (see p. 270), tyrosimenia (see p. 269), and alkaptonuria (see p. 274) as well as the condition of albinism (see p. 273).

E. Amino acids that form succinyl coenzyme A: methionine

Methionine is one of four amino acids that form succinyl CoA. This sulfur-containing amino acid deserves special attention because it is converted to S-adenosylmethionine (SAM), the major methyl-group donor in one-carbon metabolism (Figure 20.8). Methionine is also the source of homocysteine, a metabolite associated with atherosclerotic vascular disease and thrombosis (see p. 265).

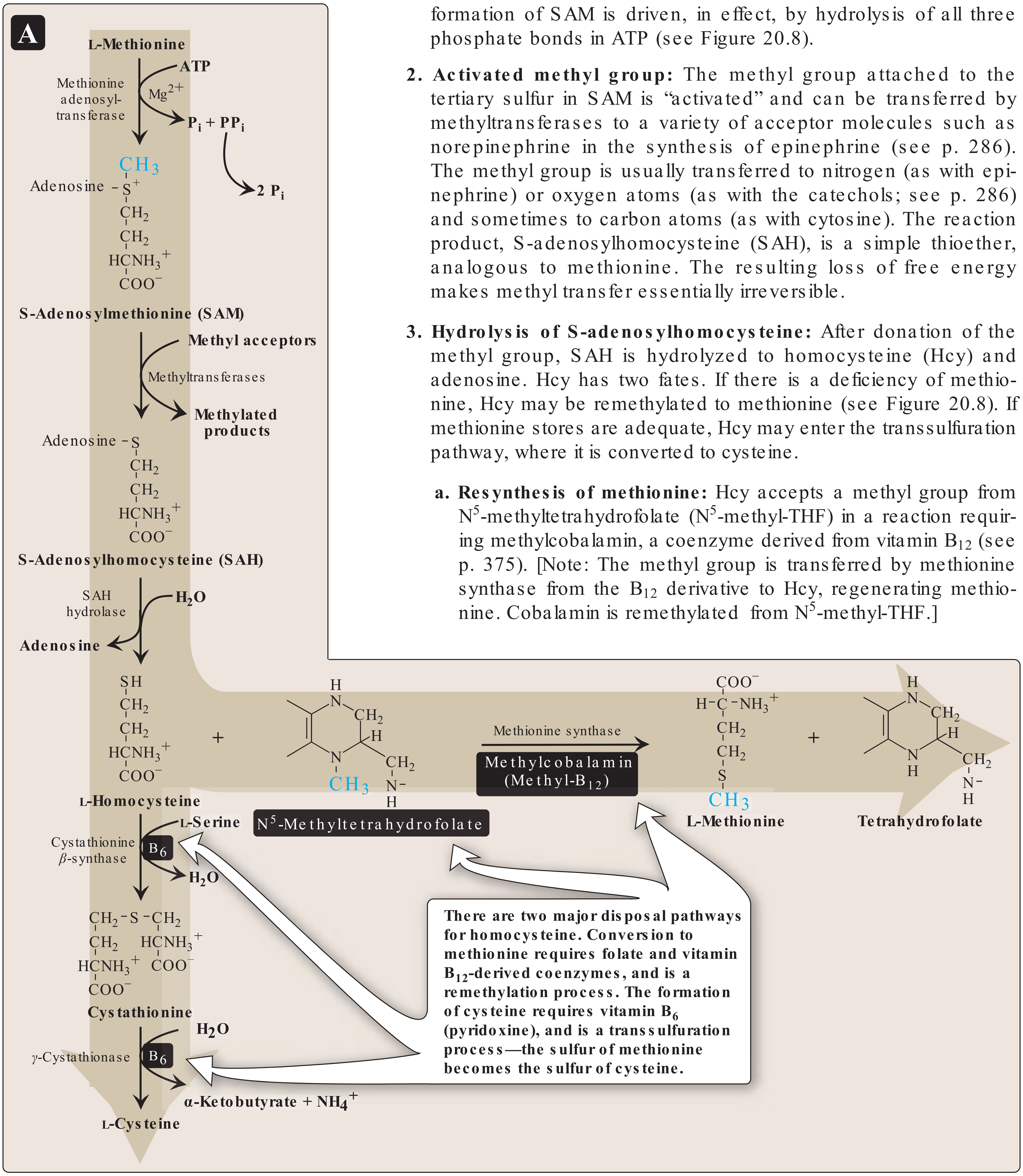
1. **Synthesis of S-adenosylmethionine:** Methionine condenses with adenosine triphosphate (ATP), forming SAM, a high-energy compound that is unusual in that it contains no phosphate. The

formation of SAM is driven, in effect, by hydrolysis of all three phosphate bonds in ATP (see Figure 20.8).

2. **Activated methyl group:** The methyl group attached to the tertiary sulfur in SAM is “activated” and can be transferred by methyltransferases to a variety of acceptor molecules such as norepinephrine in the synthesis of epinephrine (see p. 286). The methyl group is usually transferred to nitrogen (as with epinephrine) or oxygen atoms (as with the catechols; see p. 286) and sometimes to carbon atoms (as with cytosine). The reaction product, S-adenosylhomocysteine (SAH), is a simple thioether, analogous to methionine. The resulting loss of free energy makes methyl transfer essentially irreversible.

3. **Hydrolysis of S-adenosylhomocysteine:** After donation of the methyl group, SAH is hydrolyzed to homocysteine (Hcy) and adenosine. Hcy has two fates. If there is a deficiency of methionine, Hcy may be remethylated to methionine (see Figure 20.8). If methionine stores are adequate, Hcy may enter the transsulfuration pathway, where it is converted to cysteine.

   a. **Resynthesis of methionine:** Hcy accepts a methyl group from N^5-methyltetrahydrofolate (N^5-methyl-THF) in a reaction requiring methylcobalamin, a coenzyme derived from vitamin B_{12} (see p. 375). [Note: The methyl group is transferred by methionine synthase from the B_{12} derivative to Hcy, regenerating methionine. Cobalamin is remethylated from N^5-methyl-THF.]


A
L-Methionine
ATP
Methionine adenosyl-transferase
Mg^{2+}
$P_i + PP_i$
$2\ P_i$
CH_3
Adenosine $-S^+$
CH_2
CH_2
$HCNH_3^+$
COO^-
S-Adenosylmethionine (SAM)
Methyl acceptors
Methyltransferases
Methylated products
Adenosine $-S$
CH_2
CH_2
$HCNH_3^+$
COO^-
S-Adenosylhomocysteine (SAH)
SAH hydrolase
H_2O
Adenosine
SH
CH_2
CH_2
$HCNH_3^+$
COO^-
L-Homocysteine
+
N^5-Methyltetrahydrofolate
Methionine synthase
Methylcobalamin (Methyl-B_{12})
COO^-
$H-C-NH_3^+$
CH_2
CH_2
S
CH_3
L-Methionine
+
Tetrahydrofolate
L-Serine
Cystathionine β-synthase
B_6
H_2O
CH_2-S-CH_2
CH_2 $HCNH_3^+$
$HCNH_3^+$ COO^-
COO^-
Cystathionine
H_2O
γ-Cystathionase
B_6
α-Ketobutyrate + NH_4^+
L-Cysteine

There are two major disposal pathways for homocysteine. Conversion to methionine requires folate and vitamin B_{12}-derived coenzymes, and is a remethylation process. The formation of cysteine requires vitamin B_6 (pyridoxine), and is a transsulfuration process—the sulfur of methionine becomes the sulfur of cysteine.

Figure 20.8

Degradation and resynthesis of methionine. [Note: The resynthesis of methionine from homocysteine is the only reaction in which tetrahydrofolate both carries and donates a methyl ($-CH_3$) group. In all other reactions, SAM is the methyl group carrier and donor.] PP_i = pyrophosphate; P_i = inorganic phosphate.

b. Synthesis of cysteine: Hcy condenses with serine, forming cystathionine, which is hydrolyzed to α-ketobutyrate and cysteine (see Figure 20.8). This vitamin B_6–requiring sequence has the net effect of converting serine to cysteine and Hcy to α-ketobutyrate, which is oxidatively decarboxylated to form propionyl CoA. Propionyl CoA is converted to succinyl CoA (see p. 194). Because Hcy is synthesized from the essential amino acid methionine, cysteine is not an essential amino acid as long as sufficient methionine is available.

4. **Relationship of homocysteine to vascular disease:** Elevations in plasma Hcy levels promote oxidative damage, inflammation, and endothelial dysfunction and are an independent risk factor for occlusive vascular disease (Figure 20.9). Mild elevations are seen in about 7% of the population. Epidemiologic studies have shown that plasma Hcy levels are inversely related to plasma levels of folate, B_{12}, and B_6, the three vitamins involved in the conversion of Hcy to methionine or cysteine. Supplementation with these vitamins has been shown to reduce circulating levels of Hcy. However, in patients with established cardiovascular disease, vitamin therapy does not decrease cardiovascular events or death. This raises the question as to whether Hcy is a cause of the vascular damage or merely a marker of such damage. [Note: Large elevations in plasma Hcy as a result of rare deficiencies in cystathionine *β*-synthase of the transsulfuration pathway are seen in patients with classic homocystinuria. These individuals experience premature vascular disease, with about 25% dying from thrombotic complications before age 30 years.] Deficiencies in the remethylation reaction also result in a rise in Hcy.

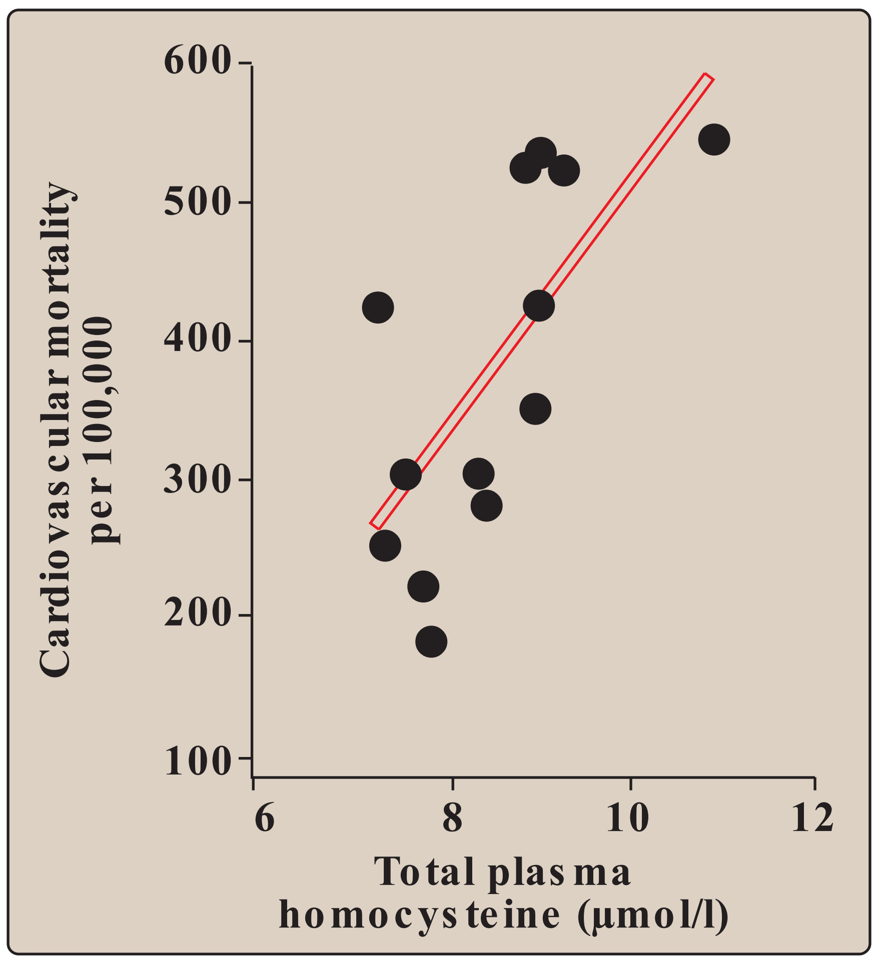


Figure 20.9
Association between cardiovascular disease mortality and total plasma homocysteine.

> Elevated homocysteine and decreased folic acid levels in pregnant women are associated with increased incidence of neural tube defects (improper closure, as in spina bifida) in the fetus. Periconceptual supplementation with folate reduces the risk of such defects.

F. Other amino acids that form succinyl coenzyme A

Degradation of valine, isoleucine, and threonine also results in the production of succinyl CoA, a TCA cycle intermediate and glucogenic compound.

1. **Valine and isoleucine:** These amino acids are branched-chain amino acids (BCAAs) that generate propionyl CoA, which is converted to methylmalonyl CoA and then succinyl CoA by biotin- and vitamin B_{12}–requiring reactions (Figure 20.10).

2. **Threonine:** This amino acid is dehydrated to α-ketobutyrate, which is converted to propionyl CoA and then to succinyl CoA. Propionyl CoA, then, is generated by the catabolism of the amino acids methionine, valine, isoleucine, and threonine. [Note: Propionyl CoA also is generated by the oxidation of odd-numbered fatty acids (see p. 193).]

G. Amino acids that form acetyl coenzyme A or acetoacetyl coenzyme A

Leucine, isoleucine, lysine, and tryptophan form acetyl CoA or acetoacetyl CoA directly, without pyruvate serving as an intermediate (through the pyruvate dehydrogenase reaction; see p. 109). As noted earlier, phenylalanine and tyrosine also give rise to acetoacetate during their catabolism (see Figure 20.7). Therefore, there are a total of six partly or wholly ketogenic amino acids.

1. **Leucine:** This amino acid is exclusively ketogenic in its catabolism, forming acetyl CoA and acetoacetate (see Figure 20.10). The initial steps in the catabolism of leucine are similar to those of the other BCAAs, isoleucine and valine (see below).
2. **Isoleucine:** This amino acid is both ketogenic and glucogenic, because its metabolism yields acetyl CoA and propionyl CoA. The first three steps in the metabolism of isoleucine are similar to the initial steps in the degradation of the other BCAAs, valine and leucine (see Figure 20.10).
3. **Lysine:** This amino acid is exclusively ketogenic and is unusual in that neither of its amino groups undergoes transamination as the first step in catabolism. Lysine is ultimately converted to acetoacetyl CoA.
4. **Tryptophan:** This amino acid is both glucogenic and ketogenic because its catabolism yields alanine and acetoacetyl CoA. [Note: Quinolinate from tryptophan catabolism is used in the synthesis of nicotinamide adenine dinucleotide (NAD, see p. 379).]

H. Catabolism of the branched-chain amino acids

The BCAAs isoleucine, leucine, and valine are essential amino acids. In contrast to other amino acids, they are metabolized primarily by the peripheral tissues (particularly muscle), rather than by the liver. Because these three amino acids have a similar route of catabolism, it is convenient to describe them as a group (see Figure 20.10).

1. **Transamination:** Transfer of the amino groups of all three BCAAs to α-ketoglutarate is catalyzed by a single, vitamin B_6–requiring enzyme, branched-chain amino acid aminotransferase.
2. **Oxidative decarboxylation:** Removal of the carboxyl group of the α-keto acids derived from leucine, valine, and isoleucine is catalyzed by a single multienzyme complex, branched-chain *α*-keto acid dehydrogenase (BCKD) complex. This complex uses thiamine pyrophosphate, lipoic acid, flavin adenine dinucleotide (FAD), NAD^+, and CoA as its coenzymes and produces NADH. [Note: This reaction is similar to the conversion of pyruvate to acetyl CoA by pyruvate dehydrogenase (PDH) complex (see p. 110) and the oxidation of α-ketoglutarate to succinyl CoA by *α*-ketoglutarate dehydrogenase complex (see p. 112). The Enzyme 3 (E3) component is identical in BCKD, PDH, and α-ketoglutarate dehydrogenase.]
3. **Dehydrogenation:** Oxidation of the products formed in the BCKD reaction yields α-β-unsaturated acyl CoA derivatives and $FADH_2$. This reaction is analogous to the FAD-linked dehydrogenation in the β-oxidation of fatty acids (see p. 192). [Note: Deficiency in the dehydrogenase specific for isovaleryl CoA causes neurologic problems and is associated with a "sweaty feet" odor in body fluids.]

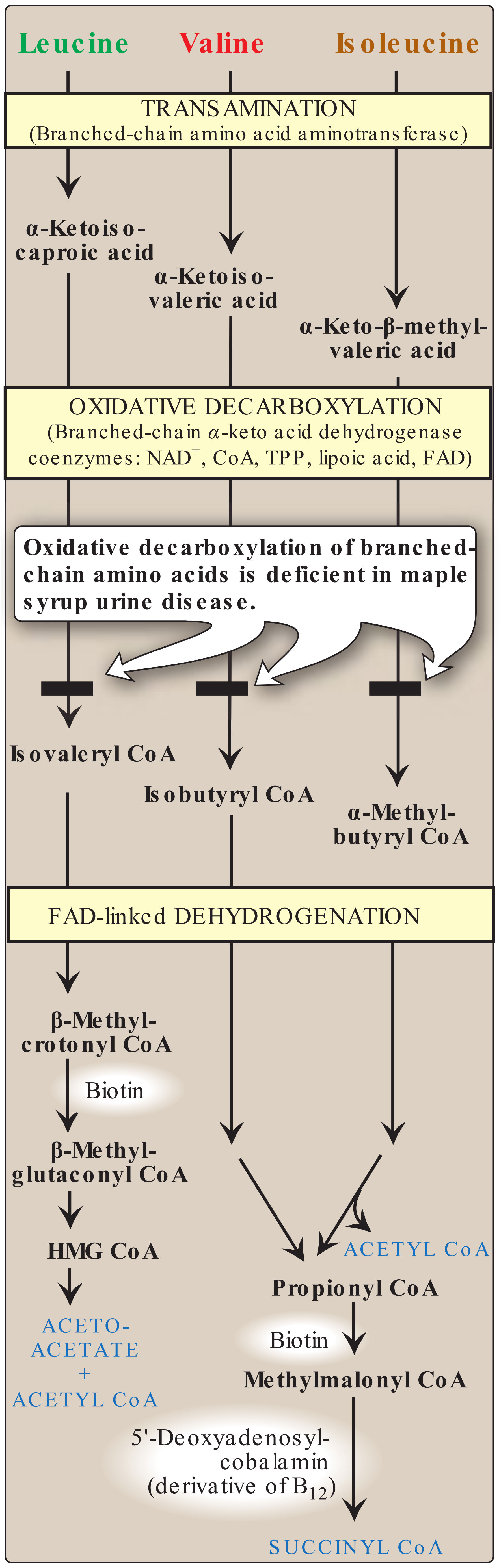

Leucine
Valine
Isoleucine
TRANSAMINATION
(Branched-chain amino acid aminotransferase)
α-Ketoisocaproic acid
α-Ketoisovaleric acid
α-Keto-β-methylvaleric acid
OXIDATIVE DECARBOXYLATION
(Branched-chain α-keto acid dehydrogenase coenzymes: NAD+, CoA, TPP, lipoic acid, FAD)
Oxidative decarboxylation of branched-chain amino acids is deficient in maple syrup urine disease.
Isovaleryl CoA
Isobutyryl CoA
α-Methylbutyryl CoA
FAD-linked DEHYDROGENATION
β-Methylcrotonyl CoA
Biotin
β-Methylglutaconyl CoA
HMG CoA
ACETOACETATE + ACETYL CoA
ACETYL CoA
Propionyl CoA
Biotin
Methylmalonyl CoA
5'-Deoxyadenosylcobalamin (derivative of B12)
SUCCINYL CoA

Figure 20.10
Degradation of leucine, valine, and isoleucine. [Note: *β*-Methylcrotonyl CoA carboxylase is one of four biotin-requiring carboxylases we have encountered. The other three are pyruvate carboxylase, acetyl CoA carboxylase, and propionyl CoA carboxylase.] TPP = thiamine pyrophosphate; FAD = flavin adenine dinucleotide; CoA = coenzyme A; NAD = nicotinamide adenine dinucleotide; HMG = hydroxymethylglutarate.

4. **End products:** The catabolism of isoleucine ultimately yields acetyl CoA and succinyl CoA, rendering it both ketogenic and glucogenic. Valine yields succinyl CoA and is glucogenic. Leucine is ketogenic, being metabolized to acetoacetate and acetyl CoA. In addition, NADH and FADH$_2$ are produced in the decarboxylation and dehydrogenation reactions, respectively. [Note: BCAA catabolism also results in glutamine and alanine being sent out into the blood from muscle (see p. 253).]

IV. FOLIC ACID AND AMINO ACID METABOLISM

Some synthetic pathways require the addition of single carbon groups that exist in a variety of oxidation states, including formyl, methenyl, methylene, and methyl. These single carbon groups can be transferred from carrier compounds such as THF and SAM to specific structures that are being synthesized or modified. The "one-carbon pool" refers to the single carbon units attached to this group of carriers. [Note: CO$_2$, the dehydrated form of carbonic acid, is carried by the vitamin biotin, which is a prosthetic group for most carboxylation reactions but is not considered a member of the one-carbon pool. Defects in the ability to add or remove biotin from carboxylases result in multiple carboxylase deficiency. Treatment is supplementation with biotin.]

A. Folic acid and one-carbon metabolism

The active form of folic acid, THF, is produced from folate by dihydrofolate reductase in a two-step reaction requiring two nicotinamide adenine dinucleotide phosphates (NADPH). The one-carbon unit carried by THF is bound to nitrogen N^5 or N^{10} or to both N^5 and N^{10}. Figure 20.11 shows the structures of the various members of the THF family and their interconversions and indicates the sources of the one-carbon units and the synthetic reactions in which the specific members participate. [Note: Folate deficiency presents as a megaloblastic anemia due to decreased availability of the purines and of the thymidine monophosphate needed for DNA synthesis (see p. 303).]

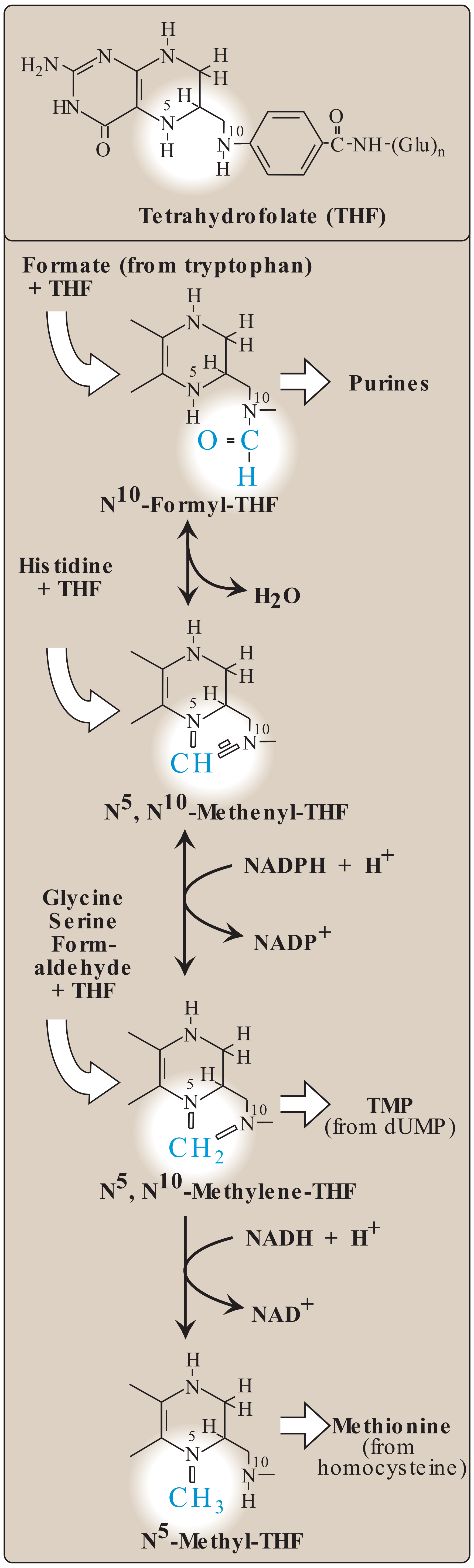


Figure 20.11
Summary of the interconversions and uses of the carrier tetrahydrofolate. [Note: N^5, N^{10}-Methenyl-THF also arises from N^5-formimino-THF (see Figure 20.4).] NADP(H) = nicotinamide adenine dinucleotide phosphate; NAD(H) = nicotinamide adenine dinucleotide; TMP = thymidine monophosphate; dUMP = deoxyuridine monophosphate.

V. BIOSYNTHESIS OF NONESSENTIAL AMINO ACIDS

Nonessential amino acids are synthesized from intermediates of metabolism or, as in the case of tyrosine and cysteine, from the essential amino acids phenylalanine and methionine, respectively. The synthetic reactions for the nonessential amino acids are described below and are summarized later in Figure 20.14. [Note: Some amino acids found in proteins, such as hydroxyproline and hydroxylysine (see p. 45), are modified after their incorporation into the protein (posttranslational modification; see p. 443).]

A. Synthesis from α-keto acids

Alanine, aspartate, and glutamate are synthesized by transfer of an amino group to the α-keto acids pyruvate, oxaloacetate, and α-ketoglutarate, respectively. These transamination reactions (Figure 20.12; also see p. 250) are the most direct of the biosynthetic pathways. Glutamate is unusual in that it can also be synthesized by the reverse of oxidative deamination, catalyzed by glutamate dehydrogenase, when ammonia levels are high (see p. 252).

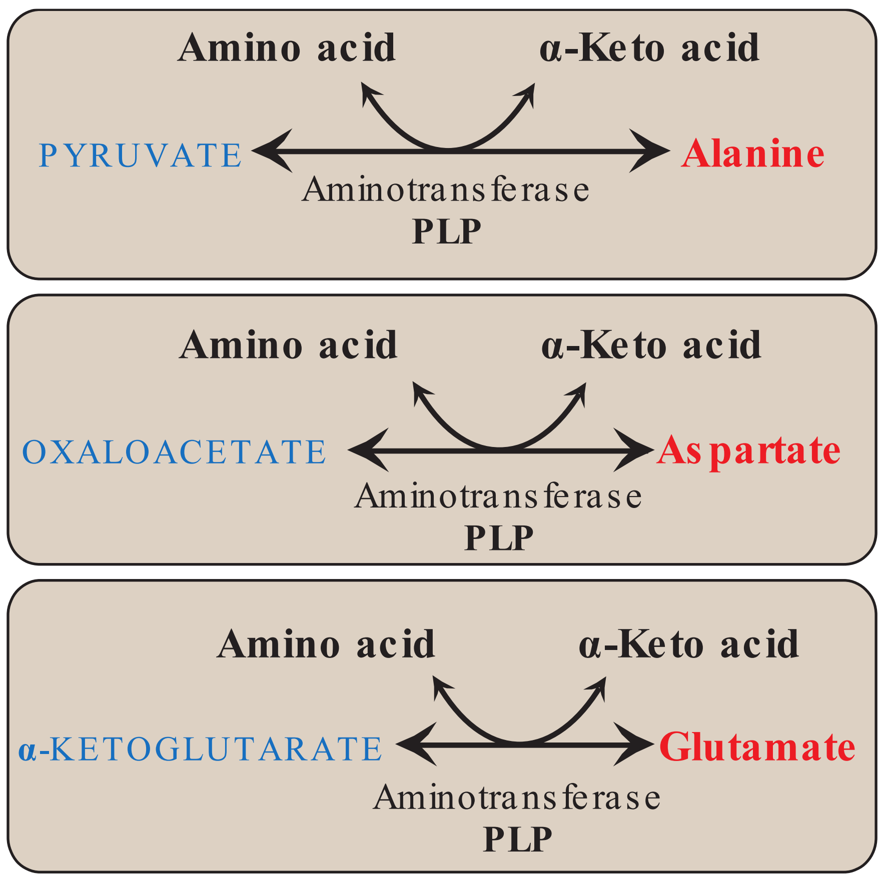


Figure 20.12
Formation of alanine, aspartate, and glutamate from the corresponding α-keto acids. PLP = pyridoxal phosphate.

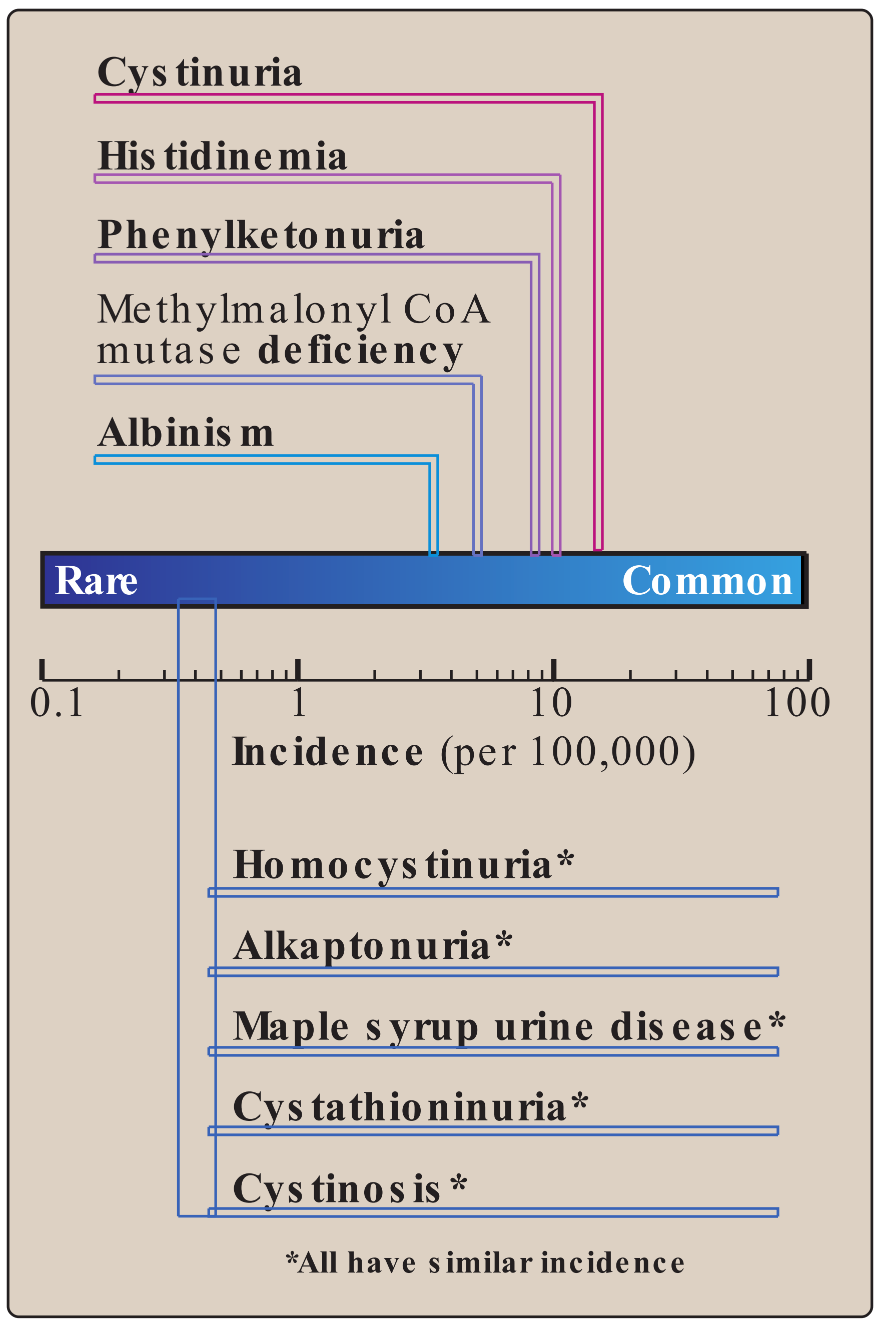


Figure 20.13
Incidence of inherited diseases of amino acid metabolism. [Note: Cystinuria is the most common genetic error of amino acid transport.]

B. Synthesis by amidation

1. **Glutamine:** This amino acid, which contains an amide linkage with ammonia at the γ-carboxyl, is formed from glutamate by glutamine synthetase (see Figure 19.18, p. 256). The reaction is driven by the hydrolysis of ATP. In addition to producing glutamine for protein synthesis, the reaction also serves as a major mechanism for the transport of ammonia in a nontoxic form (see p. 256 for a discussion of ammonia metabolism).

2. **Asparagine:** This amino acid, which contains an amide linkage with ammonia at the β-carboxyl, is formed from aspartate by asparagine synthetase, using glutamine as the amide donor. Like the synthesis of glutamine, the reaction requires ATP and has an equilibrium far in the direction of amide synthesis.

C. Proline

Glutamate via glutamate semialdehyde is converted to proline by cyclization and reduction reactions. [Note: The semialdehyde can also be transaminated to ornithine.]

D. Serine, glycine, and cysteine

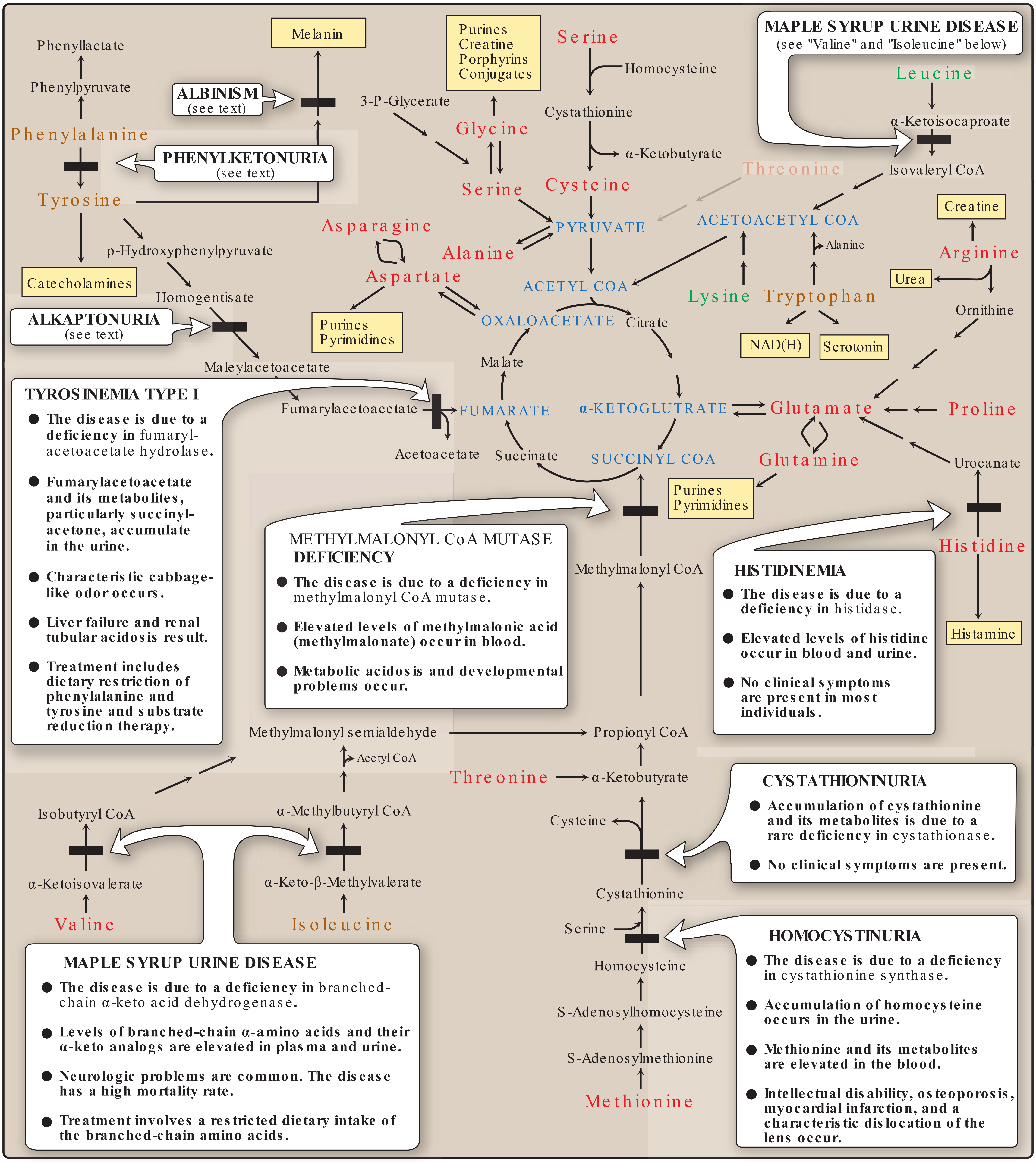
1. **Serine:** This amino acid arises from 3-phosphoglycerate, an intermediate in glycolysis (see Figure 8.18, p. 101), which is first oxidized to 3-phosphopyruvate and then transaminated to 3-phosphoserine. Serine is formed by hydrolysis of the phosphate ester. Serine can also be formed from glycine through transfer of a hydroxymethyl group by serine hydroxymethyltransferase using N^5,N^{10}-methylene-THF as the one-carbon donor (see Figure 20.6A). [Note: Selenocysteine (Sec), the 21st genetically encoded amino acid, is synthesized from serine and selenium while serine is attached to transfer RNA. Sec is found in several proteins such as glutathione peroxidase (see p. 148).]

2. **Glycine:** This amino acid is synthesized from serine by removal of a hydroxymethyl group, also by serine hydroxymethyltransferase (see Figure 20.6A). THF is the one-carbon acceptor.

3. **Cysteine:** This amino acid is synthesized by two consecutive reactions in which Hcy combines with serine, forming cystathionine, which, in turn, is hydrolyzed to α-ketobutyrate and cysteine (see Figure 20.8). (Hcy is derived from methionine as described on p. 264.) Because methionine is an essential amino acid, cysteine synthesis can be sustained only if the dietary intake of methionine is adequate.

E. Tyrosine

Tyrosine is formed from phenylalanine by phenylalanine hydroxylase. The reaction requires molecular oxygen and the coenzyme tetrahydrobiopterin (BH_4), which is synthesized from guanosine triphosphate. One atom of molecular oxygen becomes the hydroxyl group of tyrosine, and the other atom is reduced to water. During the reaction, BH_4 is oxidized to dihydrobiopterin (BH_2). BH_4 is regenerated from BH_2 by NADH-requiring dihydropteridine reductase. Tyrosine, like cysteine, is formed from an essential amino acid and is, therefore, nonessential only in the presence of adequate dietary phenylalanine.


Phenyllactate
Phenylpyruvate
Phenylalanine
Tyrosine
Melanin
ALBINISM (see text)
PHENYLKETONURIA (see text)
Catecholamines
p-Hydroxyphenylpyruvate
Homogentisate
ALKAPTONURIA (see text)
Maleylacetoacetate
Fumarylacetoacetate
Acetoacetate
FUMARATE
Purines Creatine Porphyrins Conjugates
3-P-Glycerate
Glycine
Serine
Serine
Homocysteine
Cystathionine
α-Ketobutyrate
Cysteine
PYRUVATE
Asparagine
Aspartate
Alanine
Purines Pyrimidines
ACETYL COA
OXALOACETATE
Citrate
Malate
α-KETOGLUTARATE
Succinate
SUCCINYL COA
Glutamate
Glutamine
Proline
Urocanate
Histidine
Histamine
Purines Pyrimidines
Methylmalonyl CoA
Threonine
ACETOACETYL COA
Alanine
Lysine
Tryptophan
NAD(H)
Serotonin
Leucine
α-Ketoisocaproate
Isovaleryl CoA
Creatine
Arginine
Urea
Ornithine
MAPLE SYRUP URINE DISEASE (see "Valine" and "Isoleucine" below)
Methylmalonyl semialdehyde
Acetyl CoA
Propionyl CoA
Threonine
α-Ketobutyrate
Cysteine
Cystathionine
Serine
Homocysteine
S-Adenosylhomocysteine
S-Adenosylmethionine
Methionine
Isobutyryl CoA
α-Ketoisovalerate
Valine
α-Methylbutyryl CoA
α-Keto-β-Methylvalerate
Isoleucine

TYROSINEMIA TYPE I

- **The disease is due to a deficiency in** fumarylacetoacetate hydrolase.
- **Fumarylacetoacetate and its metabolites, particularly succinylacetone, accumulate in the urine.**
- **Characteristic cabbage-like odor occurs.**
- **Liver failure and renal tubular acidosis result.**
- **Treatment includes dietary restriction of phenylalanine and tyrosine and substrate reduction therapy.**

METHYLMALONYL CoA MUTASE DEFICIENCY

- **The disease is due to a deficiency in** methylmalonyl CoA mutase.
- **Elevated levels of methylmalonic acid (methylmalonate) occur in blood.**
- **Metabolic acidosis and developmental problems occur.**

HISTIDINEMIA

- **The disease is due to a deficiency in** histidase.
- **Elevated levels of histidine occur in blood and urine.**
- **No clinical symptoms are present in most individuals.**

CYSTATHIONINURIA

- **Accumulation of cystathionine and its metabolites is due to a rare deficiency in** cystathionase.
- **No clinical symptoms are present.**

MAPLE SYRUP URINE DISEASE

- **The disease is due to a deficiency in** branched-chain α-keto acid dehydrogenase.
- **Levels of branched-chain α-amino acids and their α-keto analogs are elevated in plasma and urine.**
- **Neurologic problems are common. The disease has a high mortality rate.**
- **Treatment involves a restricted dietary intake of the branched-chain amino acids.**

HOMOCYSTINURIA

- **The disease is due to a deficiency in** cystathionine synthase.
- **Accumulation of homocysteine occurs in the urine.**
- **Methionine and its metabolites are elevated in the blood.**
- **Intellectual disability, osteoporosis, myocardial infarction, and a characteristic dislocation of the lens occur.**

Figure 20.14

Summary of the metabolism of amino acids in humans. Genetically determined enzyme deficiencies are summarized in white boxes. Nitrogen-containing compounds derived from amino acids are shown in small, yellow boxes. Classification of amino acids is color coded: **Red** = glucogenic; **brown** = glucogenic and ketogenic; **green** = ketogenic. Compounds in BLUE ALL CAPS are the seven metabolites to which all amino acid metabolism converges. CoA = coenzyme A; NAD(H) = nicotinamide adenine dinucleotide.

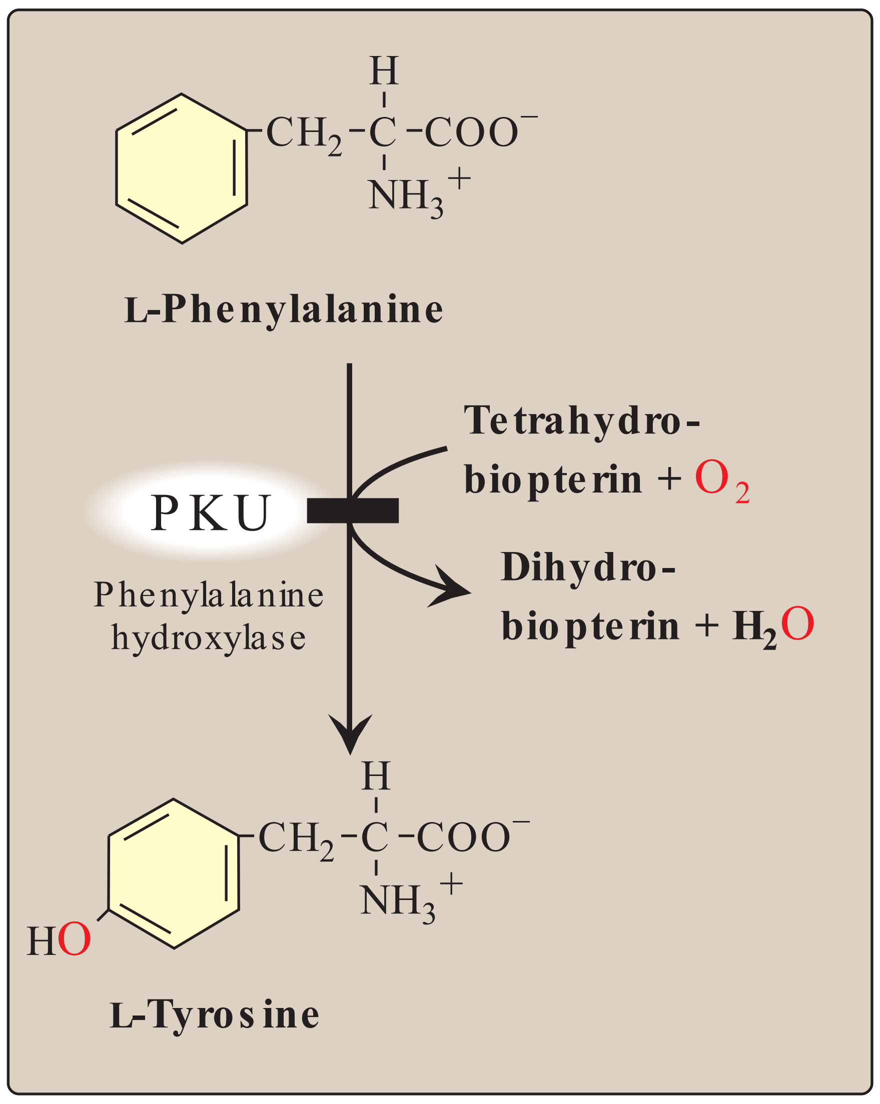

L-Phenylalanine
PKU
Phenylalanine hydroxylase
Tetrahydro-biopterin + O_2
Dihydro-biopterin + H_2O
HO
L-Tyrosine

Figure 20.15
A deficiency in phenylalanine hydroxylase results in the disease phenylketonuria (PKU).

VI. METABOLIC DEFECTS IN AMINO ACID METABOLISM

Inborn errors of metabolism are commonly caused by mutant genes that generally result in abnormal proteins, most often enzymes. The inherited defects may be expressed as a total loss of enzyme activity or, more frequently, as a partial deficiency in catalytic activity. Without treatment, the inherited defects of amino acid metabolism almost invariably result in intellectual disability or other developmental abnormalities as a consequence of harmful accumulation of metabolites. Although more than 50 of these disorders have been described, many are rare, occurring in less than 1 per 250,000 in most populations (Figure 20.13). Collectively, however, they constitute a very significant portion of pediatric genetic diseases (Figure 20.14). Phenylketonuria (PKU) is an important disease of amino acid metabolism because it is relatively common and responds to dietary treatment.

> Screening of newborns for a number of treatable disorders, including those of amino acid metabolism, is done by tandem mass spectrometry of blood obtained from a heel prick. By law, all states must screen for over 20 disorders, with some screening for over 30. All states screen for PKU.

A. Hyperphenylalanemia and phenylketonuria

PKU, caused by a deficiency of phenylalanine hydroxylase ([PAH] Figure 20.15), is the most common clinically encountered inborn error of amino acid metabolism (incidence 1:15,000). Biochemically, it is characterized by accumulation of phenylalanine, resulting in hyperphenylalanemia, and a deficiency of tyrosine. It is treated by dietary restriction of phenylalanine. Hyperphenylalanemia may also be caused by deficiencies in any of the several enzymes required to synthesize BH_4 or in dihydropteridine reductase, which regenerates BH_4 from BH_2 (Figure 20.16). Such deficiencies indirectly

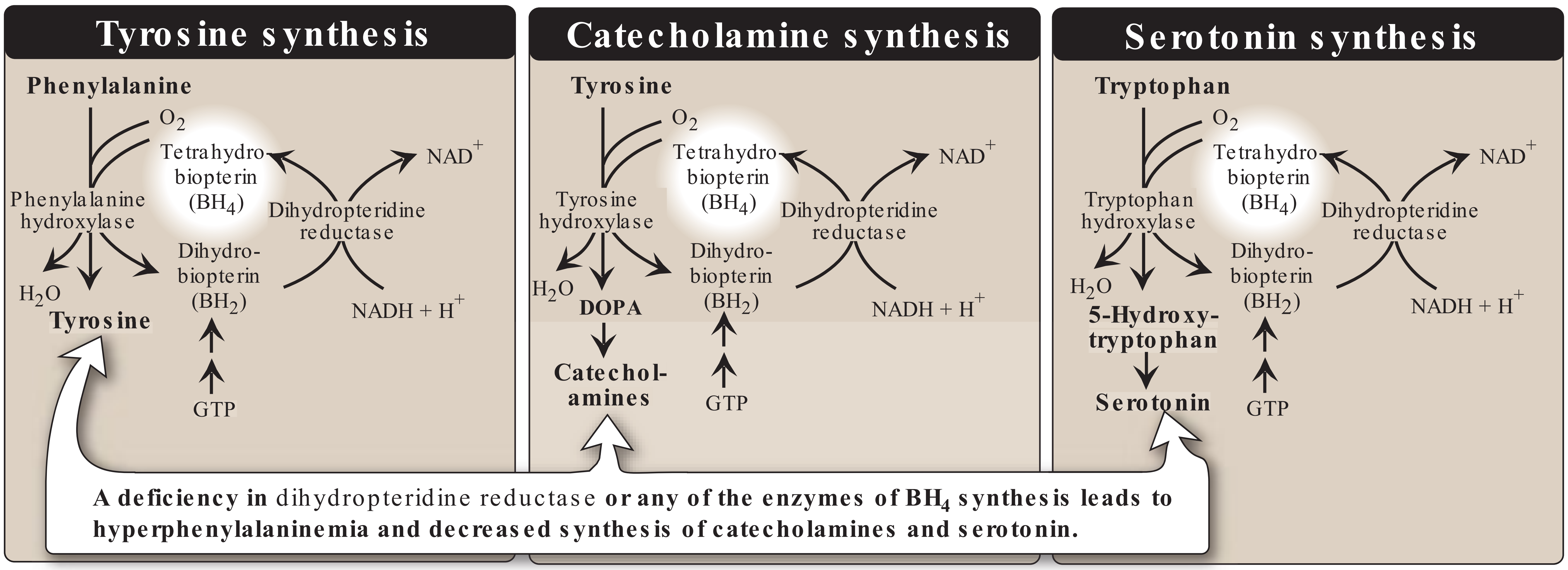

Tyrosine synthesis
Phenylalanine
O_2
Tetrahydro-biopterin (BH_4)
Phenylalanine hydroxylase
Dihydropteridine reductase
NAD^+
Dihydro-biopterin (BH_2)
H_2O
Tyrosine
$NADH + H^+$
GTP
Catecholamine synthesis
Tyrosine
O_2
Tetrahydro-biopterin (BH_4)
Tyrosine hydroxylase
Dihydropteridine reductase
NAD^+
Dihydro-biopterin (BH_2)
H_2O
DOPA
$NADH + H^+$
Catechol-amines
GTP
Serotonin synthesis
Tryptophan
O_2
Tetrahydro-biopterin (BH_4)
Tryptophan hydroxylase
Dihydropteridine reductase
NAD^+
Dihydro-biopterin (BH_2)
H_2O
5-Hydroxy-tryptophan
$NADH + H^+$
Serotonin
GTP
A deficiency in dihydropteridine reductase or any of the enzymes of BH_4 synthesis leads to hyperphenylalaninemia and decreased synthesis of catecholamines and serotonin.

Figure 20.16
Biosynthetic reactions involving amino acids and tetrahydrobiopterin. [Note: Aromatic amino acid hydroxylases use BH_4 and not PLP (pyridoxal phosphate).] NAD(H) = nicotinamide adenine dinucleotide; GTP = guanosine triphosphate; DOPA = 3,4-dihydroxyphenylalanine.

raise phenylalanine concentrations, because PAH requires BH_4 as a coenzyme. BH_4 is also required for tyrosine hydroxylase and tryptophan hydroxylase, which catalyze reactions leading to the synthesis of neurotransmitters, such as serotonin and the catecholamines. Simply restricting dietary phenylalanine does not reverse the central nervous system effects due to deficiencies in neurotransmitters. Supplementation with BH_4 and replacement therapy with L-3,4-dihydroxyphenylalanine and 5-hydroxytryptophan (products of the affected tyrosine hydroxylase– and tryptophan hydroxylase–catalyzed reactions) improves the clinical outcome in these variant forms of hyperphenylalaninemia, although the response is unpredictable.

1. **Characteristics of classic phenylketonuria:**
   a. **Elevated phenylalanine:** Phenylalanine is present in high concentrations (ten times normal) in tissues, plasma, and urine. Phenyllactate, phenylacetate, and phenylpyruvate, which are not normally produced in significant amounts in the presence of functional PAH, are also elevated in PKU (Figure 20.17). These metabolites give urine a characteristic musty ("mousey") odor. [Note: The disease acquired its name from the presence of a phenylpyruvate, a phenylketone in the urine.]

   b. **Central nervous system symptoms:** Severe intellectual disability, developmental delay, microcephaly, and seizures are characteristic findings in untreated PKU. The patient with untreated PKU typically shows symptoms of intellectual disability by age 1 year and rarely achieves an intelligence quotient (IQ) greater than 50 (Figure 20.18). [Note: These clinical manifestations are now rarely seen as a result of neonatal screening programs.]

   c. **Hypopigmentation:** Patients with untreated PKU may show a deficiency of pigmentation (fair hair, light skin color, and blue eyes). The hydroxylation of tyrosine by copper-requiring tyrosinase, which is the first step in the formation of the pigment melanin, is inhibited in PKU.

2. **Neonatal screening and diagnosis:** Early diagnosis of PKU is important because the disease is treatable by dietary means. Due to the lack of neonatal symptoms, laboratory testing for elevated blood levels of phenylalanine is mandatory for detection. However, the infant with PKU frequently has normal blood levels of phenylalanine at birth because the mother clears increased blood phenylalanine in her affected fetus through the placenta. Normal levels of phenylalanine may persist until the newborn is exposed to 24–48 hours of protein feeding. Thus, screening tests are typically done after this time to avoid false negatives. For newborns with a positive screening test, diagnosis is confirmed through quantitative determination of phenylalanine levels.

3. **Prenatal diagnosis:** Classic PKU is caused by any of 100 or more different mutations in the gene that codes for PAH. The frequency of any given mutation varies among populations, and the disease is often doubly heterozygous (that is, the PAH gene has a different mutation in each allele). Despite this complexity, prenatal diagnosis is possible (see p. 477).

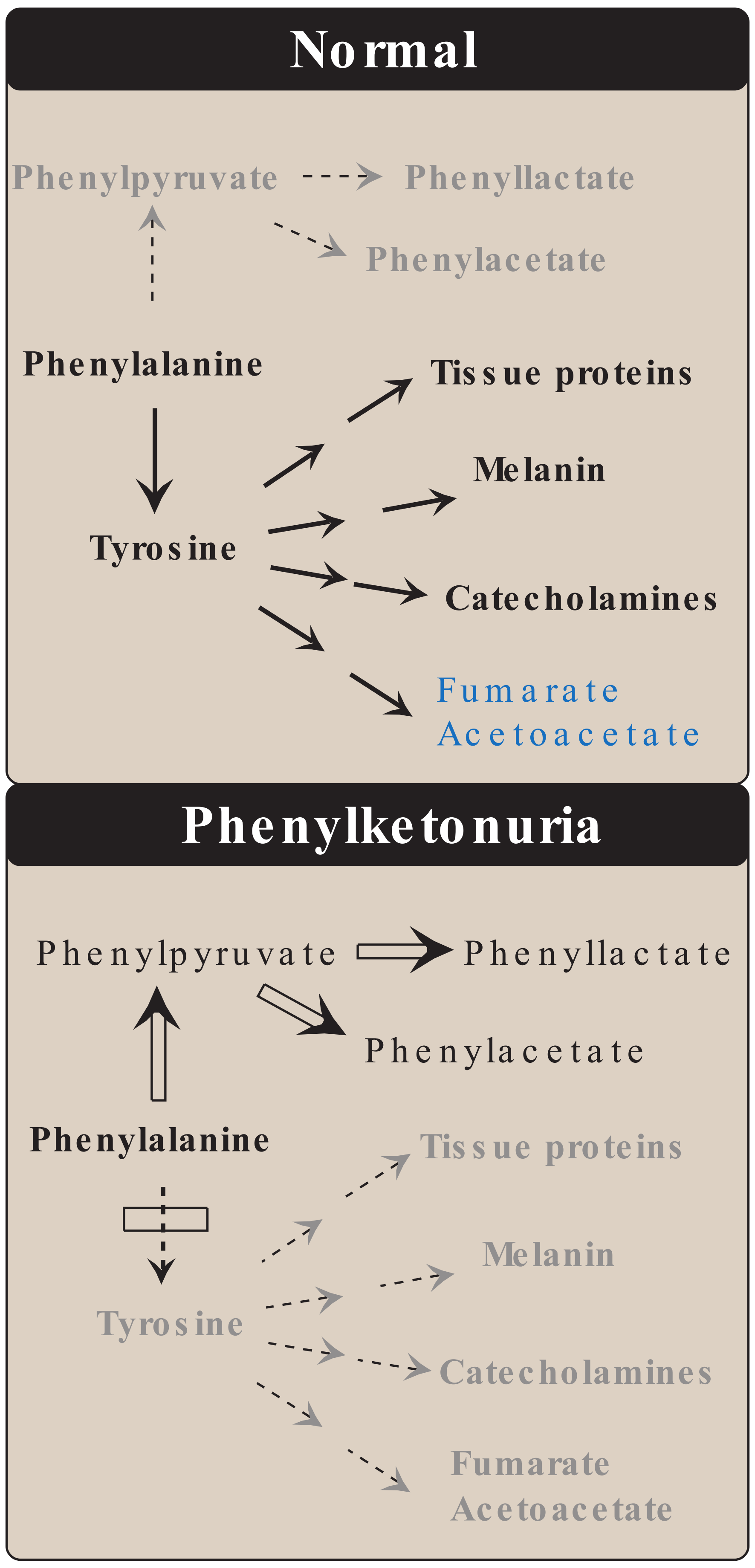


Figure 20.17
Pathways of phenylalanine metabolism in normal individuals and in patients with phenylketonuria.

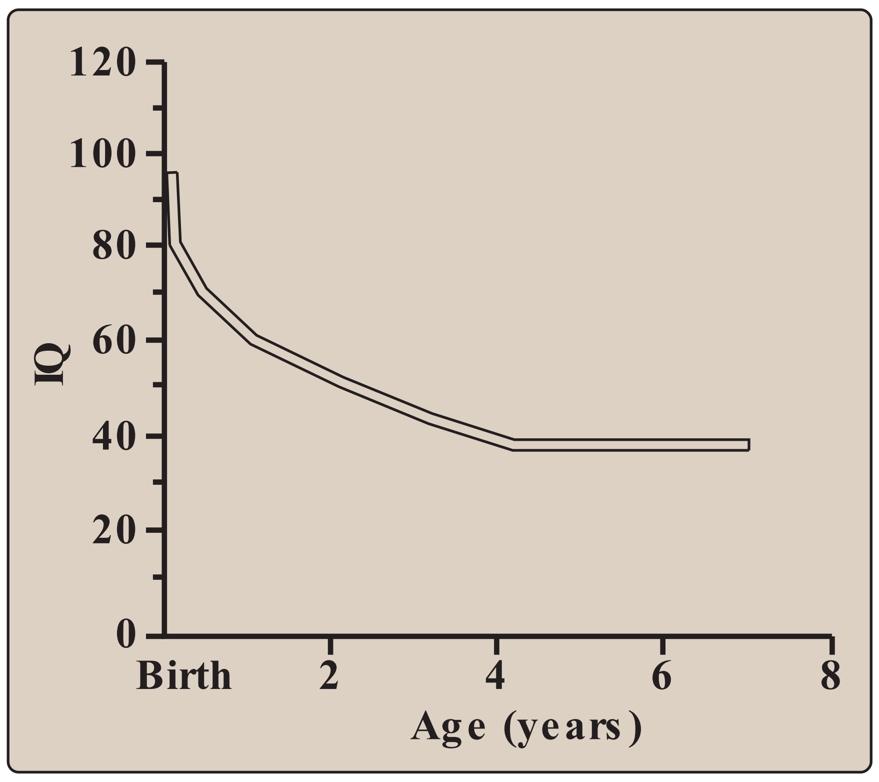


Figure 20.18
Typical intellectual ability in untreated patients of different ages with phenylketonuria.
IQ = intelligence quotient.

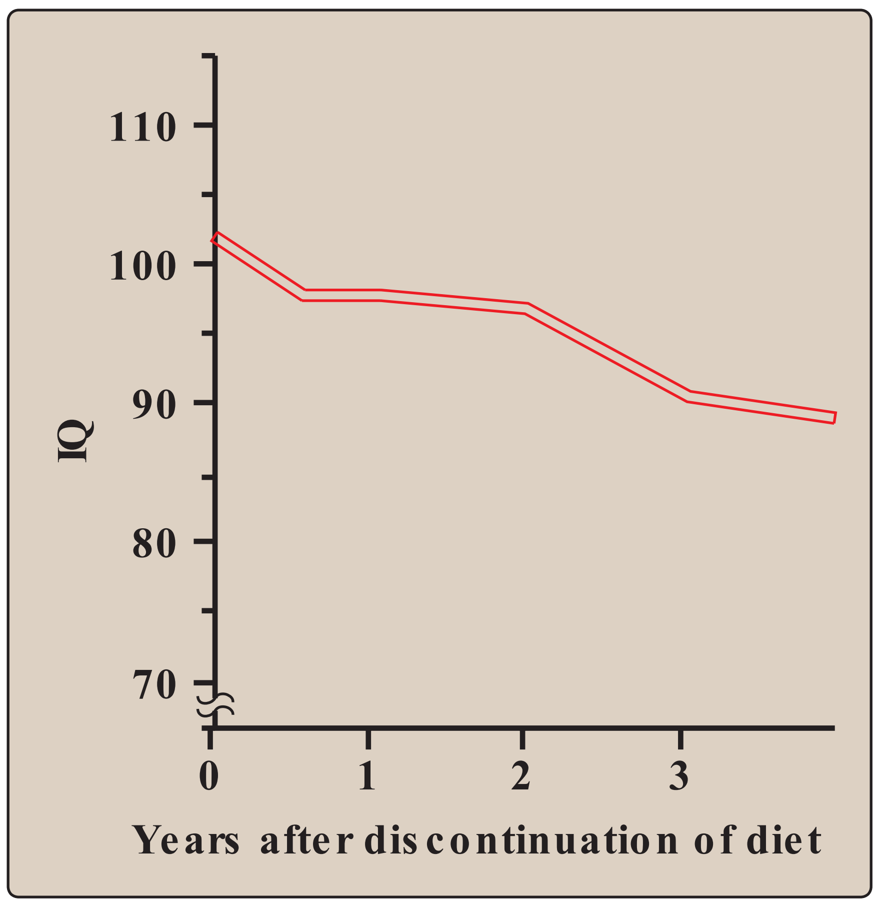


Figure 20.19
Changes in IQ scores after discontinuation of low-phenylalanine diet in patients with phenylketonuria. IQ = intelligence quotient.

4. **Treatment:** Most natural protein contains phenylalanine, an essential amino acid, and it is impossible to satisfy the body's protein requirement without exceeding the phenylalanine limit when ingesting a normal diet. Therefore, in PKU, blood phenylalanine level is maintained close to the normal range by feeding synthetic amino acid preparations free of phenylalanine, supplemented with some natural foods (such as fruits, vegetables, and certain cereals) selected for their low phenylalanine content. The amount is adjusted according to the tolerance of the individual as measured by blood phenylalanine levels. The earlier treatment is started, the more completely neurologic damage can be prevented. Individuals who are appropriately treated can have normal intelligence. [Note: Treatment must begin during the first 7–10 days of life to prevent cognitive impairment.] Because phenylalanine is an essential amino acid, overzealous treatment that results in blood phenylalanine levels below normal is avoided. In patients with PKU, tyrosine cannot be synthesized from phenylalanine, and, therefore, it becomes an essential amino acid and must be supplied in the diet. Discontinuance of the phenyalanine-restricted diet in early childhood is associated with poor performance on IQ tests. Adult PKU patients show deterioration of IQ scores after discontinuation of the diet (Figure 20.19). Lifelong restriction of dietary phenylalanine is, therefore, recommended. [Note: Individuals with PKU are advised to avoid aspartame, an artificial sweetener that contains phenylalanine.]

5. **Maternal phenylketonuria:** If women with PKU who are not on a low-phenylalanine diet become pregnant, the offspring are affected with "maternal PKU syndrome." High blood phenylalanine levels in the mother cause microcephaly and congenital heart abnormalities in the fetus (phenylalanine is a teratogen). Because these developmental responses to high phenylalanine occur during the first months of pregnancy, dietary control of blood phenylalanine must begin prior to conception and must be maintained throughout the pregnancy.

B. Maple syrup urine disease

Maple syrup urine disease (MSUD) is a rare (1:185,000), autosomal recessive disorder in which there is a partial or complete deficiency in BCKD, a mitochondrial enzyme complex that oxidatively decarboxylates leucine, isoleucine, and valine (see Figure 20.10). These BCAAs and their corresponding α-keto acids accumulate in the blood, causing a toxic effect that interferes with brain functions. The disease is characterized by feeding problems, vomiting, ketoacidosis, changes in muscle tone, neurologic problems that can result in coma (primarily due to the rise in leucine), and a characteristic maple syrup-like odor of the urine due to the rise in isoleucine. If untreated, the disease is fatal. If treatment is delayed, intellectual disability results.

1. **Classification:** The term "maple syrup urine disease" includes a classic type and several variant forms of the disorder. The classic, neonatal-onset form is the most common type of MSUD. Leukocytes or cultured skin fibroblasts from these patients show little or no BCKD activity. Infants with classic MSUD show symptoms within the first several days of life. If not diagnosed and

treated, classic MSUD is lethal in the first weeks of life. Patients with intermediate forms have a higher level of enzyme activity (up to 30% of normal). The symptoms are milder and show an onset from infancy to adolescence. Patients with the rare thiamine-dependent variant of MSUD respond to large doses of this vitamin.

2. **Screening and diagnosis:** As with PKU, prenatal diagnosis and neonatal screening are available, and most affected individuals are compound heterozygotes.

3. **Treatment:** MSUD is treated with a synthetic formula that is free of BCAAs, supplemented with limited amounts of leucine, isoleucine, and valine to allow for normal growth and development without producing toxic levels. [Note: Elevated leucine is the cause of the neurologic damage in MSUD, and its level is carefully monitored.] Early diagnosis and lifelong dietary treatment is essential if the child with MSUD is to develop normally. [Note: BCAAs are an important energy source in times of metabolic need, and individuals with MSUD are at risk of decompensation during periods of increased protein catabolism.]

C. Albinism

Albinism refers to a group of conditions in which a defect in tyrosine metabolism results in a deficiency in the production of melanin. These defects result in the partial or full absence of pigment from the skin, hair, and eyes. Albinism appears in different forms, and it may be inherited by one of several modes: autosomal recessive (primary mode), autosomal dominant, or X linked. Total absence of pigment from the hair, eyes, and skin (Figure 20.20), tyrosinase-negative oculocutaneous albinism (type 1 albinism), results from an absent or defective copper-requiring tyrosinase. It is the most severe form of the condition. In addition to hypopigmentation, affected individuals have vision defects and photophobia (sunlight hurts their eyes). They are at increased risk for skin cancer.

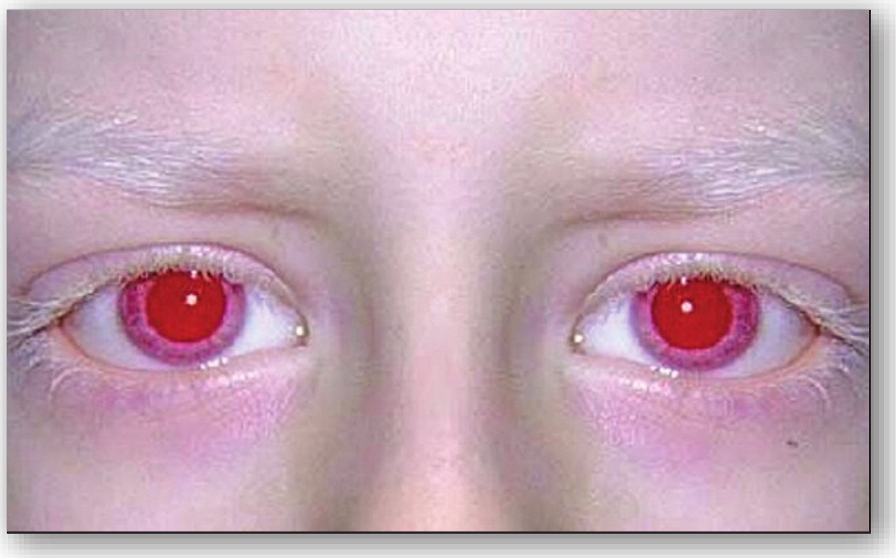


Figure 20.20
Patient with oculocutaneous albinism, showing white eyebrows and lashes and eyes that appear red in color.

D. Homocystinuria

The homocystinurias are a group of disorders involving defects in the metabolism of Hcy. These autosomal-recessive diseases are characterized by high plasma and urinary levels of Hcy and methionine and low levels of cysteine. The most common cause of homocystinuria is a defect in the enzyme cystathionine β-synthase, which converts Hcy to cystathionine (Figure 20.21). Individuals who are homozygous for cystathionine β-synthase deficiency exhibit dislocation of the lens (ectopia lentis), skeletal anomalies (long limbs and fingers), intellectual disability, and an increased risk for developing thrombi (blood clots). Thrombosis is the major cause of early death in these individuals. Patients can be responsive or nonresponsive to oral administration of pyridoxine (vitamin B_6), which is converted to pyridoxal phosphate, the coenzyme of cystathionine β-synthase. Vitamin B_6–responsive patients usually have a milder and later onset of clinical symptoms compared with B_6-nonresponsive patients. Treatment includes restriction of methionine intake and supplementation with vitamins B_6, B_{12}, and folate.

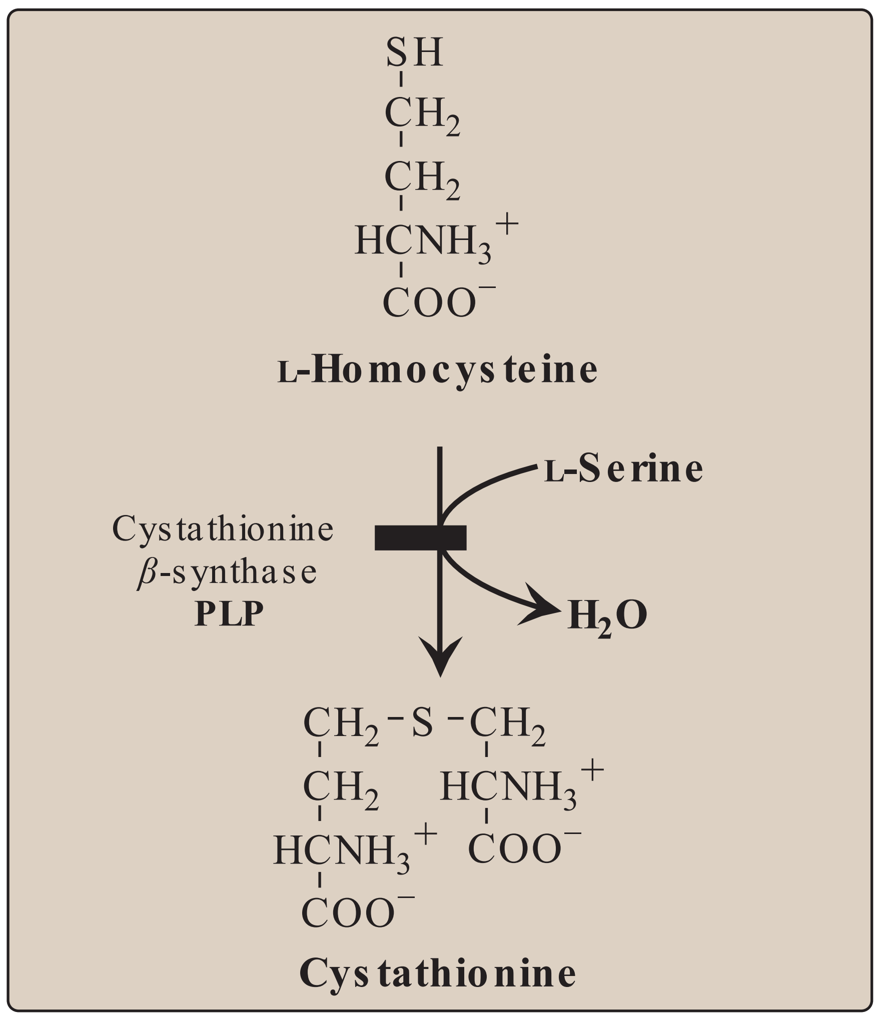


Figure 20.21
Enzyme deficiency in homocystinuria. PLP = pyridoxal phosphate.

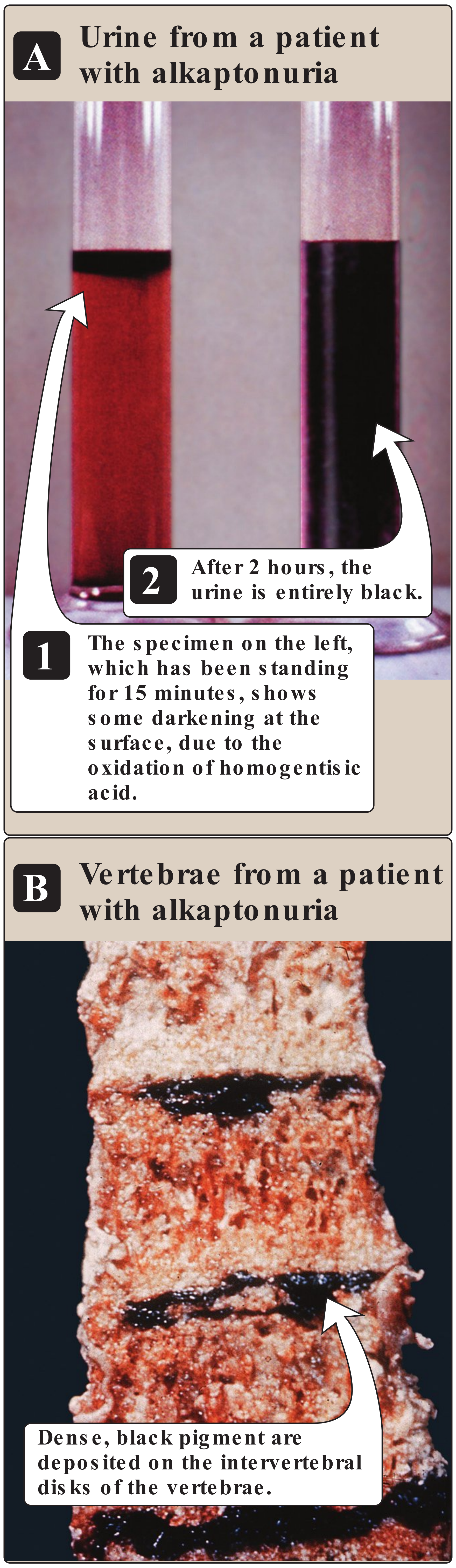

A
Urine from a patient with alkaptonuria
2
After 2 hours, the urine is entirely black.
1
The specimen on the left, which has been standing for 15 minutes, shows some darkening at the surface, due to the oxidation of homogentisic acid.
B
Vertebrae from a patient with alkaptonuria
Dense, black pigment are deposited on the intervertebral disks of the vertebrae.

Figure 20.22
A patient with alkaptonuria.
A. Urine. B. Vertebrae.

E. Alkaptonuria (alcaptonuria)

Alkaptonuria is a rare metabolic condition involving a deficiency in homogentisic acid oxidase, resulting in the accumulation of homogentisic acid (HA), an intermediate in the degradative pathway of tyrosine (see p. 269.) The condition has three characteristic symptoms: homogentisic aciduria (the urine contains elevated levels of HA, which is oxidized to a dark pigment on standing, as shown in Figure 20.22A), large joint arthritis, and deposition of black pigment (ochronosis) in cartilage and collagenous tissue (Figure 20.22B). Patients with alkaptonuria are usually asymptomatic until about age 40 years. Dark staining of diapers can indicate the disease in infants, but usually no symptoms are present until later in life. Diets low in phenylalanine and tyrosine reduce the levels of HA and decrease the amount of pigment deposited in body tissues. Although alkaptonuria is not life-threatening, the associated arthritis may be severely crippling.

VII. CHAPTER SUMMARY

Amino acids whose catabolism yields **pyruvate** or one of the **intermediates of the tricarboxylic acid cycle** are termed **glucogenic** (Figure 20.23).They can give rise to the net formation of **glucose** in the **liver** and the **kidney**. The solely glucogenic amino acids are glutamine, glutamate, proline, arginine, histidine, alanine, serine, glycine, cysteine, methionine, valine, threonine, aspartate, and asparagine. Amino acids whose catabolism yields either **acetoacetate** or one of its precursors, **acetyl coenzyme A** (**CoA**) or **acetoacetyl CoA**, are termed **ketogenic**. Leucine and lysine are solely ketogenic. Tyrosine, phenylalanine, tryptophan, and isoleucine are both ketogenic and glucogenic. **Nonessential amino acids** can be synthesized from metabolic intermediates or from the carbon skeletons of essential amino acids. **Essential amino acids** need to be obtained from the **diet**. They include histidine, methionine, threonine, valine, isoleucine, phenylalanine, tryptophan, leucine, and lysine. **Phenylketonuria** (**PKU**) is caused by a **deficiency** of phenylalanine hydroxylase (PAH), the enzyme that converts phenylalanine to tyrosine. **Hyperphenylalaninemia** may also be caused by deficiencies in the enzymes that synthesize or regenerate the coenzyme for PAH, **tetrahydrobiopterin**. Untreated patients with PKU suffer from severe intellectual disability, developmental delay, microcephaly, seizures and a characteristic mousey smell of the urine. Treatment involves controlling dietary phenylalanine. Tyrosine becomes an essential dietary component for people with PKU. **Maple syrup urine disease** is caused by a partial or complete **deficiency** in branched-chain α-keto acid dehydrogenase, the enzyme that decarboxylates **leucine**, **isoleucine**, and **valine**. Symptoms include feeding problems, vomiting, ketoacidosis, changes in muscle tone, and a characteristic sweet smell of the urine. If untreated, the disease leads to neurologic problems that result in death. Treatment involves controlling dietary leucine, isoleucine, and valine. Other important genetic diseases associated with amino acid metabolism include **albinism**, **homocystinuria**, methylmalonyl CoA mutase **deficiency**, **alkaptonuria**, **histidinemia**, **tyrosinemia**, and **cystathioninuria**.

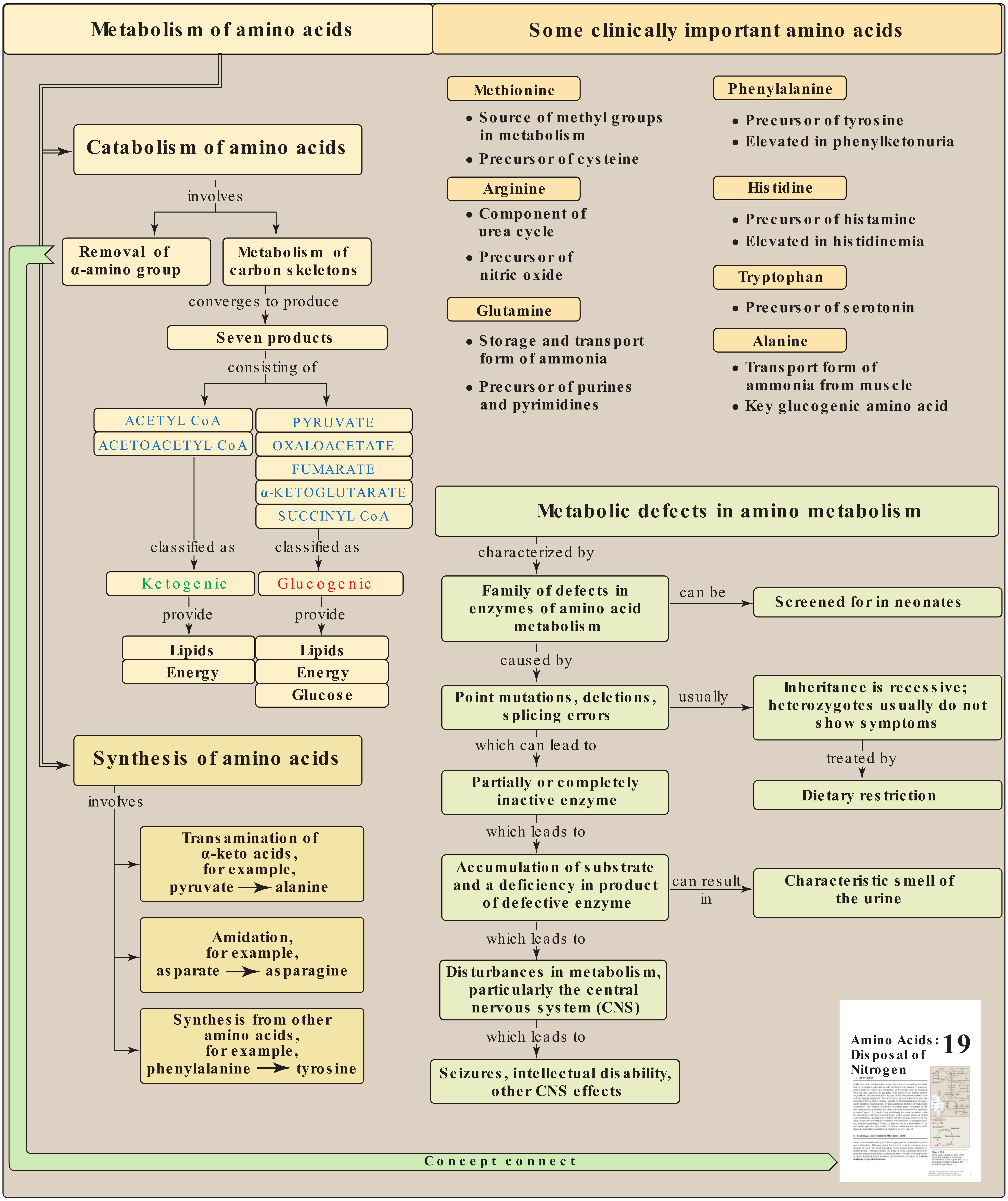

Metabolism of amino acids
Catabolism of amino acids
involves
Removal of α-amino group
Metabolism of carbon skeletons
converges to produce
Seven products
consisting of
ACETYL CoA
ACETOACETYL CoA
PYRUVATE
OXALOACETATE
FUMARATE
α-KETOGLUTARATE
SUCCINYL CoA
classified as
classified as
Ketogenic
Glucogenic
provide
provide
Lipids
Energy
Lipids
Energy
Glucose
Synthesis of amino acids
involves
Transamination of α-keto acids, for example, pyruvate → alanine
Amidation, for example, asparate → asparagine
Synthesis from other amino acids, for example, phenylalanine → tyrosine
Concept connect
Some clinically important amino acids
Methionine
• Source of methyl groups in metabolism
• Precursor of cysteine
Arginine
• Component of urea cycle
• Precursor of nitric oxide
Glutamine
• Storage and transport form of ammonia
• Precursor of purines and pyrimidines
Phenylalanine
• Precursor of tyrosine
• Elevated in phenylketonuria
Histidine
• Precursor of histamine
• Elevated in histidinemia
Tryptophan
• Precursor of serotonin
Alanine
• Transport form of ammonia from muscle
• Key glucogenic amino acid
Metabolic defects in amino metabolism
characterized by
Family of defects in enzymes of amino acid metabolism
can be
Screened for in neonates
caused by
Point mutations, deletions, splicing errors
usually
Inheritance is recessive; heterozygotes usually do not show symptoms
treated by
Dietary restriction
which can lead to
Partially or completely inactive enzyme
which leads to
Accumulation of substrate and a deficiency in product of defective enzyme
can result in
Characteristic smell of the urine
which leads to
Disturbances in metabolism, particularly the central nervous system (CNS)
which leads to
Seizures, intellectual disability, other CNS effects
Amino Acids: Disposal of Nitrogen
19

Figure 20.23
Key concept map for amino acid metabolism. CoA = coenzyme A.

Conversion of Amino Acids to Specialized Products

21

I. OVERVIEW

In addition to serving as building blocks for proteins, amino acids are precursors of many nitrogen-containing compounds that have important physiologic functions (Figure 21.1). These molecules include porphyrins, neurotransmitters, hormones, purines, and pyrimidines.

II. PORPHYRIN METABOLISM

Porphyrins are cyclic compounds that readily bind metal ions, usually ferrous (Fe^{2+}) or ferric (Fe^{3+}) iron. The most prevalent metalloporphyrin in humans is heme, which consists of one Fe^{2+} coordinated in the center of the tetrapyrrole ring of protoporphyrin IX (see p. 280). Heme is the prosthetic group for hemoglobin, myoglobin, the cytochromes, the cytochrome P450 (CYP) monooxygenase system, catalase, nitric oxide synthase, and peroxidase. These hemeproteins are rapidly synthesized and degraded. For example, 6–7 g of hemoglobin are synthesized each day to replace heme lost through the normal turnover of erythrocytes. The simultaneous synthesis and degradation of the associated porphyrins and recycling of the bound iron ions is coordinated with the turnover of hemeproteins.

A. Structure of porphyrins

Porphyrins are cyclic molecules formed by the linkage of four pyrrole rings through methenyl bridges (Figure 21.2). Three structural features of these molecules are relevant to understanding their medical significance.

1. **Side chains:** Different porphyrins vary in the nature of the side chains that are attached to each of the four pyrrole rings. Uroporphyrin contains acetate ($-CH_2-COO^-$) and propionate ($-CH_2-CH_2-COO^-$) side chains; coproporphyrin contains methyl ($-CH_3$) and propionate groups; and protoporphyrin IX (and heme) contains vinyl ($-CH{=}CH_2$), methyl, and propionate groups. [Note: The methyl and vinyl groups are produced by decarboxylation of acetate and propionate side chains, respectively.]

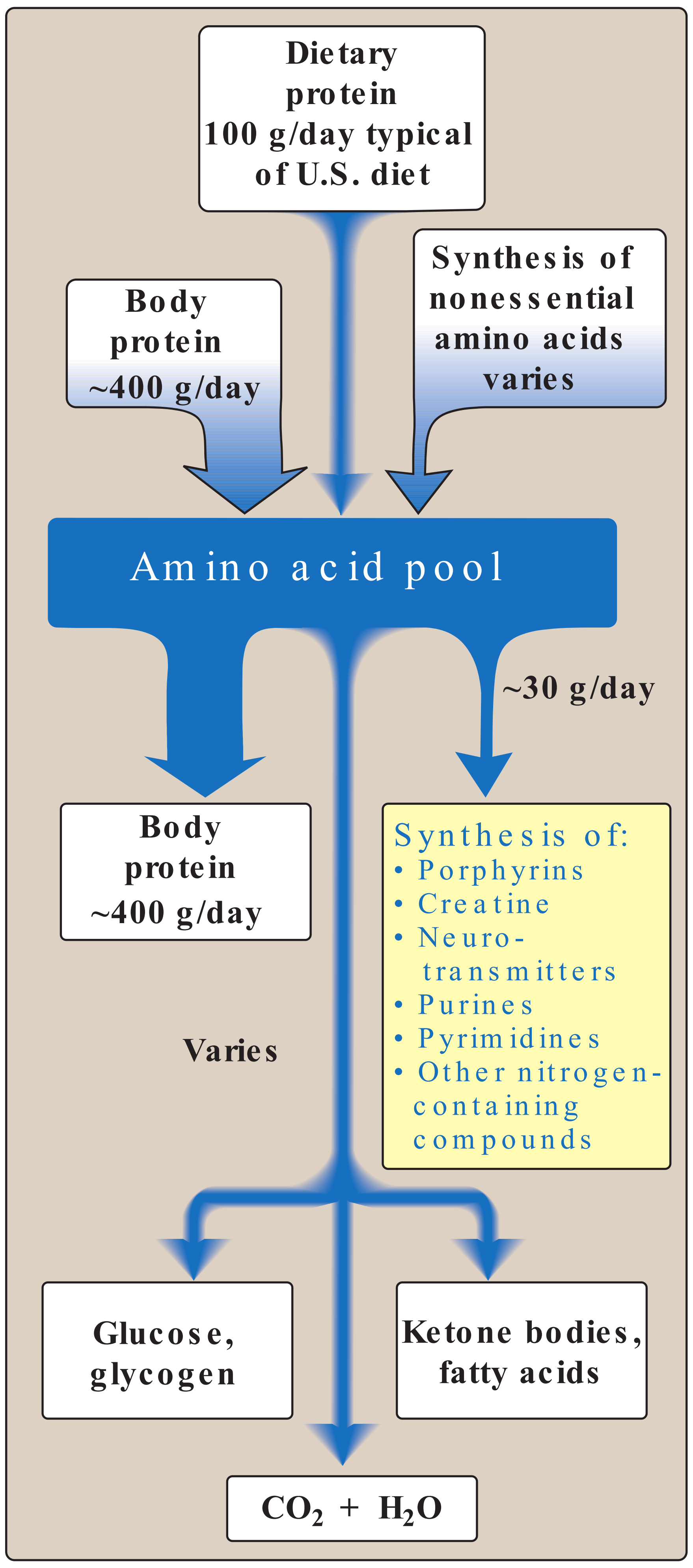
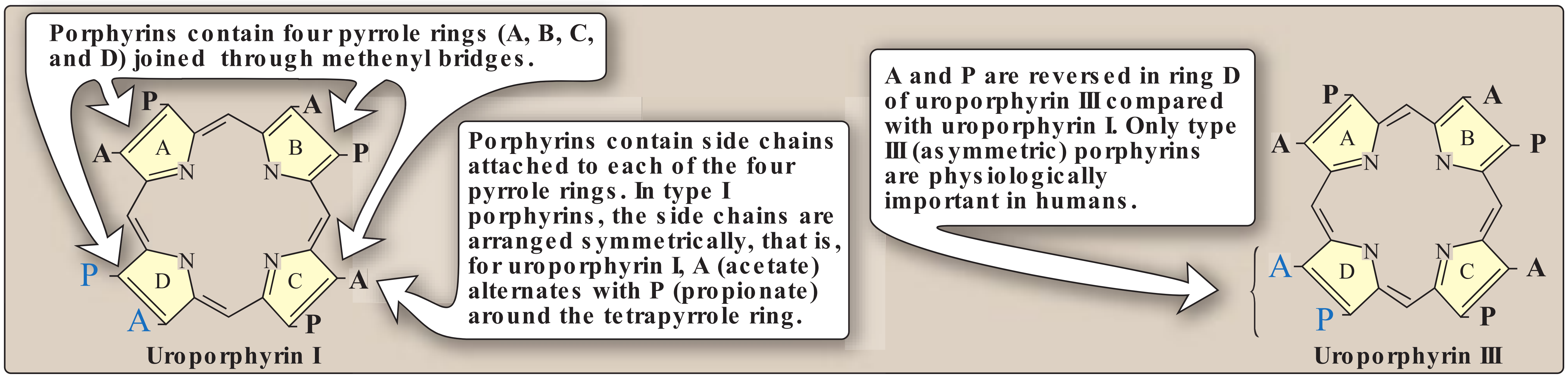

Dietary protein 100 g/day typical of U.S. diet
Body protein ~400 g/day
Synthesis of nonessential amino acids varies
Amino acid pool
~30 g/day
Body protein ~400 g/day
Synthesis of:
• Porphyrins
• Creatine
• Neurotransmitters
• Purines
• Pyrimidines
• Other nitrogen-containing compounds
Varies
Glucose, glycogen
Ketone bodies, fatty acids
CO_2 + H_2O

Figure 21.1
Amino acids as precursors of nitrogen-containing compounds.

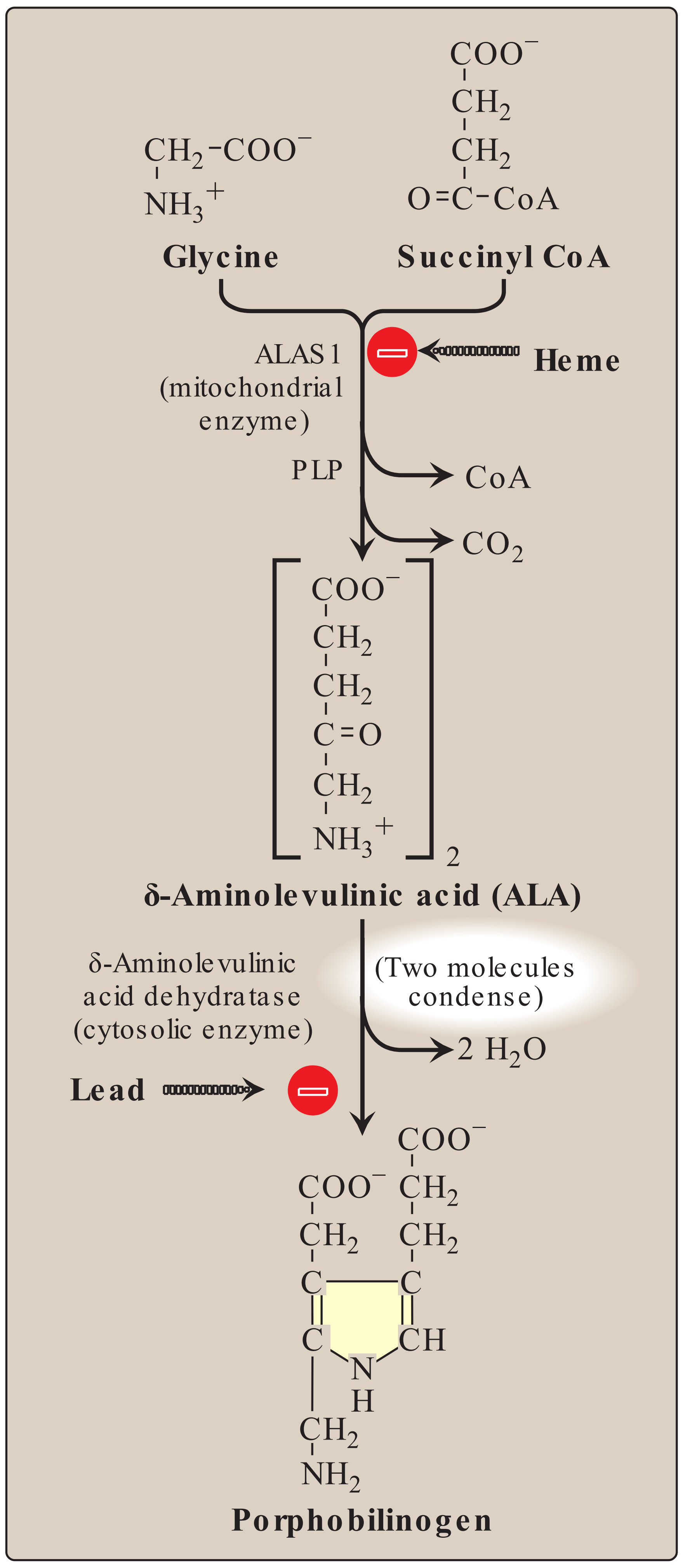


Porphyrins contain four pyrrole rings (A, B, C, and D) joined through methenyl bridges.

Porphyrins contain side chains attached to each of the four pyrrole rings. In type I porphyrins, the side chains are arranged symmetrically, that is, for uroporphyrin I, A (acetate) alternates with P (propionate) around the tetrapyrrole ring.

A and P are reversed in ring D of uroporphyrin III compared with uroporphyrin I. Only type III (asymmetric) porphyrins are physiologically important in humans.

Figure 21.2
Structures of uroporphyrin I and uroporphyrin III.



CH_2-COO^-
NH_3^+
Glycine
COO^-
CH_2
CH_2
$O=C-CoA$
Succinyl CoA
ALAS1 (mitochondrial enzyme)
Heme
PLP
CoA
CO_2
$[COO^- - CH_2 - CH_2 - C=O - CH_2 - NH_3^+]_2$
δ-Aminolevulinic acid (ALA)
δ-Aminolevulinic acid dehydratase (cytosolic enzyme)
(Two molecules condense)
$2\ H_2O$
Lead
Porphobilinogen

Figure 21.3
Pathway of porphyrin synthesis: Formation of porphobilinogen. ALAS = δ-aminolevulinic acid synthase; CoA = coenzyme A; PLP = pyridoxal phosphate. (Continued in Figures 21.4 and 21.5.)

2. **Distribution of side chains:** The side chains of porphyrins can be ordered around the tetrapyrrole nucleus in four different ways, designated by Roman numerals I to IV. Only type III porphyrins, which contain an asymmetric substitution on ring D (see Figure 21.2), are physiologically important in humans. [Note: Protoporphyrin IX is a member of the type III series.]

3. **Porphyrinogens:** These porphyrin precursors (for example, uroporphyrinogen) exist in a chemically reduced, colorless form and serve as intermediates between porphobilinogen and the oxidized, colored protoporphyrins in heme biosynthesis.

B. Biosynthesis of heme

The major sites of heme biosynthesis are the liver, which synthesizes a number of heme proteins (particularly the CYP proteins), and the erythrocyte-producing cells of the bone marrow, which are active in hemoglobin synthesis. [Note: Over 85% of all heme synthesis occurs in erythroid tissue.] In the liver, the rate of heme synthesis is highly variable, responding to alterations in the cellular heme pool caused by fluctuating demands for heme proteins. In contrast, heme synthesis in erythroid cells is relatively constant and is matched to the rate of globin synthesis. The initial reaction and the last three steps in the formation of porphyrins occur in mitochondria, whereas the intermediate steps of the biosynthetic pathway occur in the cytosol (see Figure 21.8). [Note: Mature red blood cells (RBCs) lack mitochondria and are unable to synthesize heme.]

1. **Formation of δ-aminolevulinic acid:** All the carbon and nitrogen atoms of the porphyrin molecule are provided by glycine (a nonessential amino acid) and succinyl coenzyme A (a citric acid cycle intermediate) that condense to form d-aminolevulinic acid (ALA) in a reaction catalyzed by ALA synthase ([ALAS] Figure 21.3) This reaction requires pyridoxal phosphate (PLP) as a coenzyme and is the committed and rate-limiting step in porphyrin biosynthesis. [Note: There are two isoforms of ALAS, 1 and 2, each produced by different genes and controlled by different mechanisms. ALAS1 is found in all tissues, whereas ALAS2 is erythroid specific. Loss-of-function mutations in ALAS2 result in X-linked sideroblastic anemia.]

    a. **Effect of heme (hemin):** When porphyrin production exceeds the availability of the apoproteins that require it, heme accumulates and is converted to hemin by the oxidation of Fe^{2+} to Fe^{3+}. Hemin decreases the amount (and thereby the activity) of ALAS1 by repressing transcription of its gene, increasing degradation of its messenger RNA, and decreasing import of the enzyme into mitochondria. [Note: In erythroid cells, ALAS2 is controlled by the availability of intracellular iron.]

b. Effect of drugs: Administration of any of a large number of drugs results in a significant increase in hepatic ALAS1 activity. These drugs are metabolized by the microsomal CYP monooxygenase system, a hemeprotein oxidase system found in the liver (see p. 149). In response to these drugs, the synthesis of CYP proteins increases, leading to an enhanced consumption of heme, a component of these proteins. This, in turn, causes a decrease in the concentration of heme in liver cells. The lower intracellular heme concentration leads to an increase in the synthesis of ALAS1 and prompts a corresponding increase in the synthesis of ALA.

2. **Formation of porphobilinogen:** The condensation of two molecules of ALA to form porphobilinogen by zinc-containing ALA dehydratase (porphobilinogen synthase) is extremely sensitive to inhibition by heavy metal ions (for example, lead) that replace the zinc (see Figure 21.3). This inhibition is, in part, responsible for the elevation in ALA and the anemia seen in lead poisoning.

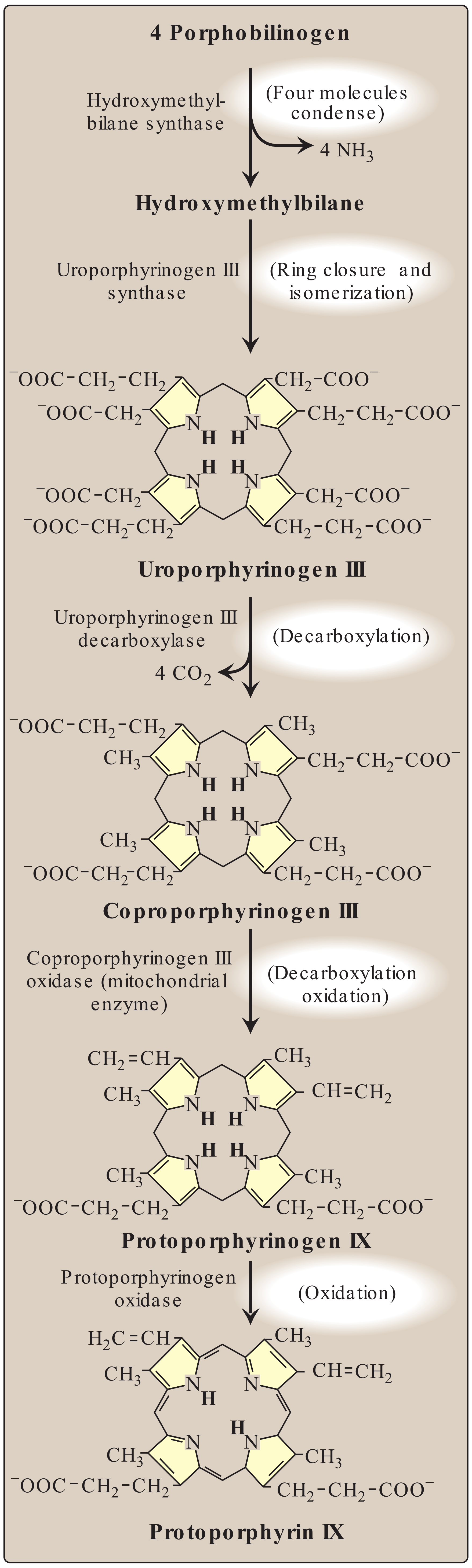
3. **Formation of uroporphyrinogen:** The condensation of four porphobilinogens produces the linear tetrapyrrole hydroxymethylbilane, which is cyclized and isomerized by uroporphyrinogen III synthase to produce the asymmetric uroporphyrinogen III. This cyclic tetrapyrrole undergoes decarboxylation of its acetate groups, generating coproporphyrinogen III (Figure 21.4). These reactions occur in the cytosol.

4. **Formation of heme:** Coproporphyrinogen III enters the mitochondrion, and two propionate side chains are decarboxylated by coproporphyrinogen III oxidase to vinyl groups generating protoporphyrinogen IX, which is oxidized to protoporphyrin IX. The introduction of iron (as Fe^{2+}) into protoporphyrin IX can occur spontaneously, but the rate is enhanced by ferrochelatase, an enzyme that, like ALA dehydratase, is inhibited by lead (Figure 21.5).

C. Porphyrias

Porphyrias are rare, inherited (or occasionally acquired) defects in heme synthesis, resulting in the accumulation and increased excretion of porphyrins or porphyrin precursors (see Figure 21.8). [Note: With few exceptions, porphyrias are inherited as autosomal-dominant disorders.] Each porphyria results in the accumulation of a unique pattern of intermediates caused by the deficiency of an enzyme in the heme synthetic pathway. [Note: "Porphyria" refers to the red-blue color caused by pigment-like porphyrins in the urine of some patients with defects in heme synthesis.]

1. **Clinical manifestations:** The porphyrias are classified as erythropoietic or hepatic, depending on whether the enzyme deficiency occurs in the erythropoietic cells of the bone marrow or in the liver. Hepatic porphyrias can be further classified as chronic or acute. In



4 Porphobilinogen
Hydroxymethylbilane synthase
(Four molecules condense)
4 NH3
Hydroxymethylbilane
Uroporphyrinogen III synthase
(Ring closure and isomerization)
⁻OOC-CH2-CH2
⁻OOC-CH2
CH2-COO⁻
CH2-CH2-COO⁻
N H H N
⁻OOC-CH2
⁻OOC-CH2-CH2
CH2-COO⁻
CH2-CH2-COO⁻
Uroporphyrinogen III
Uroporphyrinogen III decarboxylase
(Decarboxylation)
4 CO2
CH3
Coproporphyrinogen III
Coproporphyrinogen III oxidase (mitochondrial enzyme)
(Decarboxylation oxidation)
CH2=CH
CH=CH2
Protoporphyrinogen IX
Protoporphyrinogen oxidase
(Oxidation)
H2C=CH
Protoporphyrin IX

Figure 21.4
Pathway of porphyrin synthesis: formation of protoporphyrin IX. (Continued from Figure 21.3.) [Note: Deficiency in uroporphyrinogen III synthase prevents isomerization, resulting in production of type I porphyrins.]

general, individuals with an enzyme defect prior to the synthesis of the tetrapyrroles manifest abdominal and neuropsychiatric signs, whereas those with enzyme defects leading to the accumulation of tetrapyrrole intermediates show photosensitivity (that is, their skin itches and burns [pruritus] when exposed to visible light). [Note: Photosenstivity is a result of the oxidation of colorless porphyrinogens to colored porphyrins, which are photosensitizing molecules thought to participate in the formation of superoxide radicals from oxygen. These reactive oxygen species can oxidatively damage membranes and cause the release of destructive enzymes from lysosomes.]

a. **Chronic hepatic porphyria:** Porphyria cutanea tarda, the most common porphyria, is a chronic disease of the liver. The disease is associated with a deficiency in uroporphyrinogen decarboxylase, but clinical expression of the enzyme deficiency is influenced by various factors, such as hepatic iron overload, exposure to sunlight, alcohol ingestion, estrogen therapy, and the presence of hepatitis B or C or HIV infections. Clinical onset is typically during the fourth or fifth decade of life. Porphyrin accumulation leads to cutaneous symptoms (Figure 21.6) as well as urine that is red to brown in natural light (Figure 21.7) and pink to red in fluorescent light.

b. **Acute hepatic porphyrias:** Acute hepatic porphyrias (ALA dehydratase deficiency porphyria, acute intermittent porphyria, hereditary coproporphyria, and variegate porphyria) are characterized by acute attacks of gastrointestinal (GI), neuropsychiatric, and motor symptoms that may be accompanied by photosensitivity. Porphyrias leading to accumulation of ALA and porphobilinogen, such as acute intermittent porphyria, cause abdominal pain and neuropsychiatric disturbances, ranging from anxiety to delirium. Symptoms of the acute hepatic porphyrias are often precipitated by use of drugs, such as barbiturates and ethanol, which induce the synthesis of the heme-containing CYP microsomal drug oxidation system. This further decreases the amount of available heme, which, in turn, promotes increased synthesis of ALAS1.

c. **Erythropoietic porphyrias:** The chronic erythropoietic porphyrias (congenital erythropoietic porphyria and erythropoietic protoporphyria) are characterized by skin rashes and blisters that appear in early childhood. [Note: Patients with erythropoietic protoporphyria are also at risk for hepatobiliary disease.]

2. **Increased δ-aminolevulinic acid synthase activity:** One common feature of the porphyrias is a decreased synthesis of heme. In the liver, heme normally functions as a repressor of the gene for ALAS1. Therefore, the absence of this end product results in an increase in the synthesis of ALAS1 (derepression). This causes an increased synthesis of intermediates that occur prior to the genetic block. The accumulation of these toxic intermediates is the major pathophysiology of the porphyrias.

3. **Treatment:** During acute porphyria attacks, patients require medical support, particularly treatment for pain and vomiting. The severity of acute symptoms of the porphyrias can be diminished

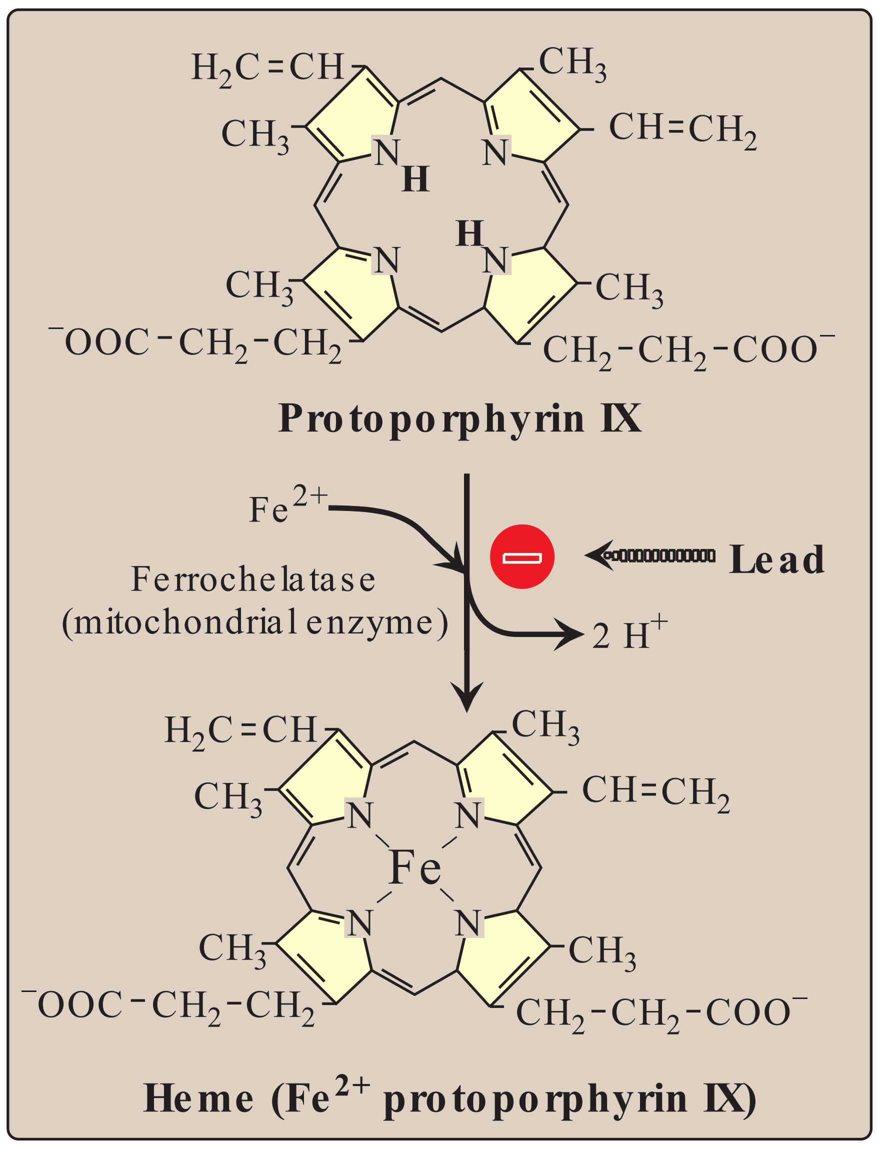

Protoporphyrin IX
Fe^{2+}
Ferrochelatase (mitochondrial enzyme)
Lead
2 H^+
Heme (Fe^{2+} protoporphyrin IX)

Figure 21.5
Pathway of porphyrin synthesis: formation of heme. (Continued from Figures 21.3 and 21.4.) Fe^{2+} = ferrous iron.

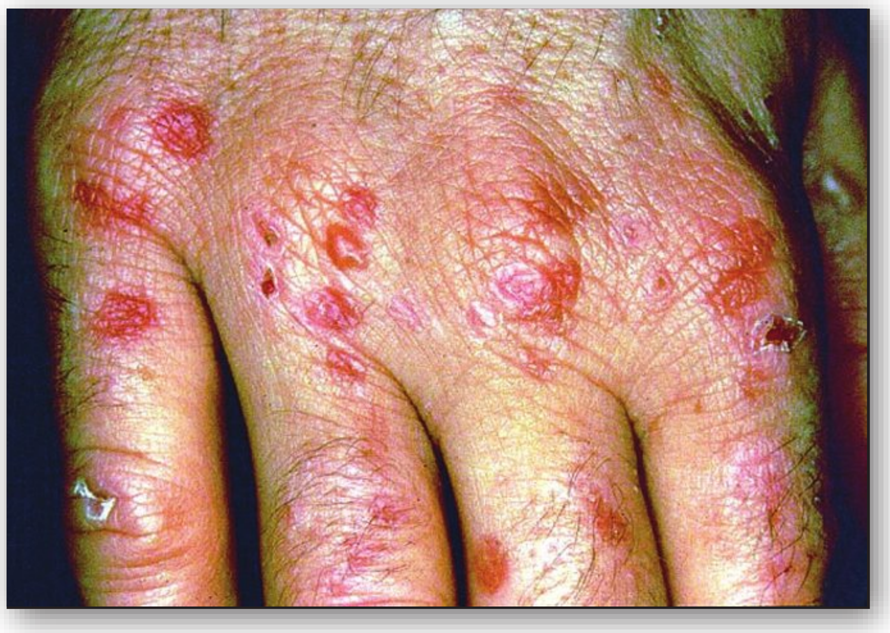


Figure 21.6
Skin eruptions in a patient with porphyria cutanea tarda.

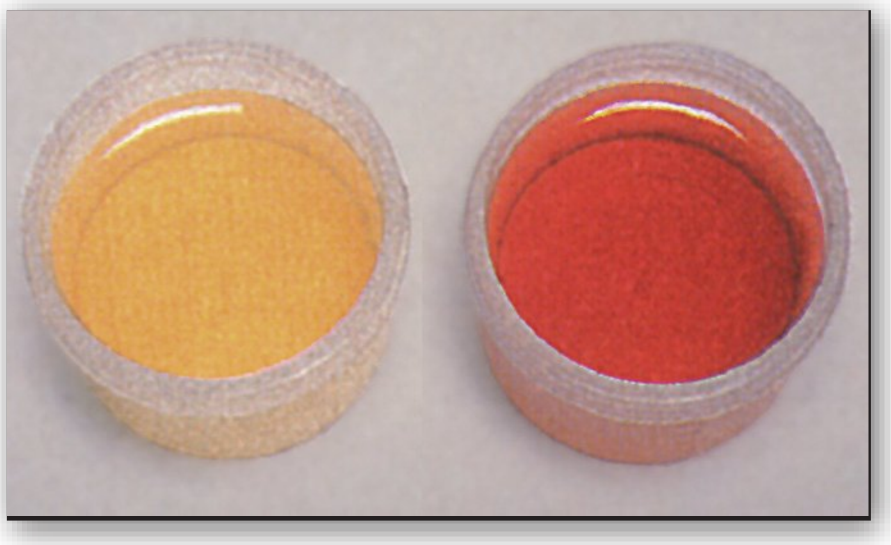
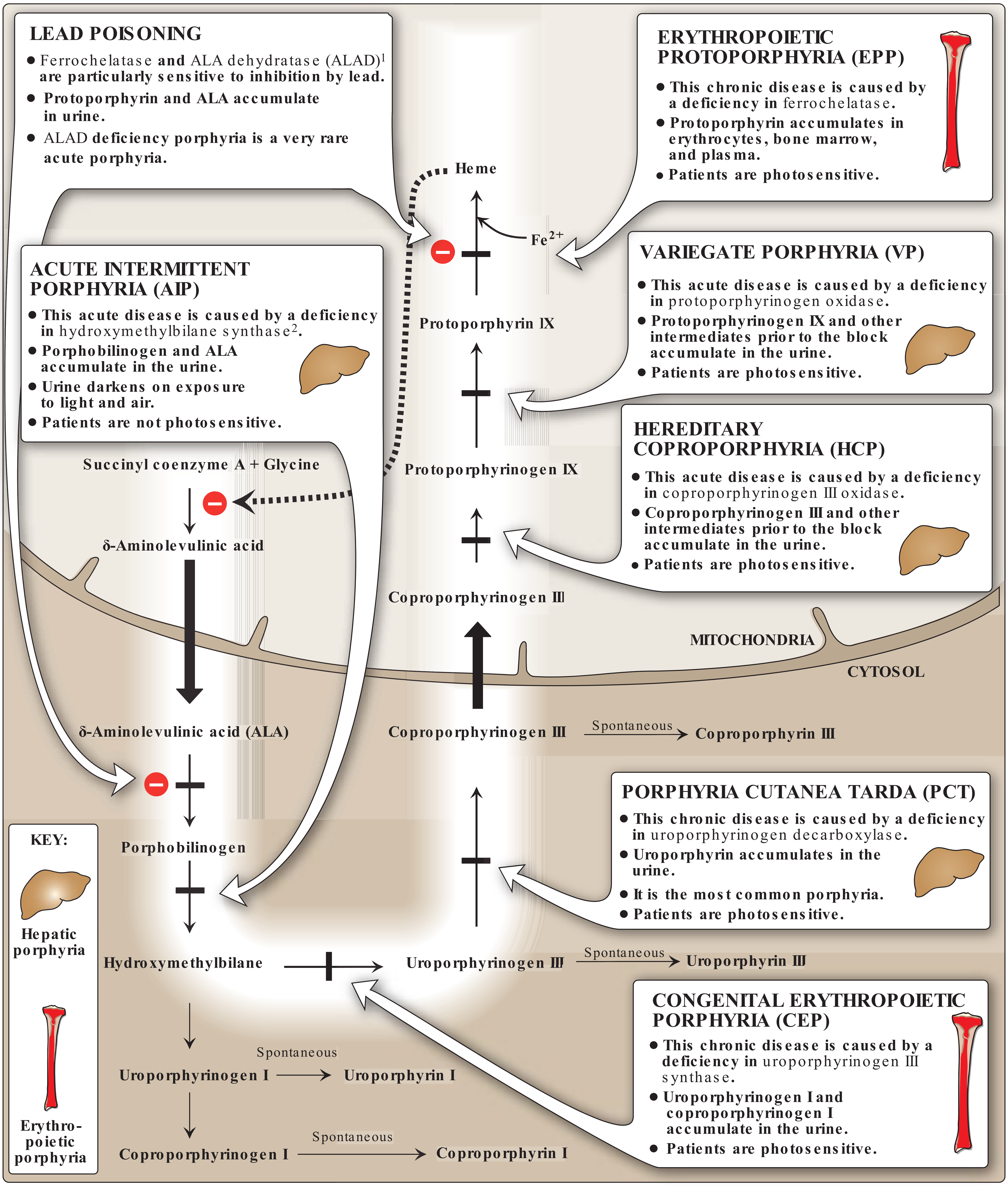


Figure 21.7
Urine from a patient with porphyria cutanea tarda (right) and from a patient with normal porphyrin excretion (left).



LEAD POISONING

- Ferrochelatase and ALA dehydratase (ALAD)[1] are particularly sensitive to inhibition by lead.
- Protoporphyrin and ALA accumulate in urine.
- ALAD deficiency porphyria is a very rare acute porphyria.

ERYTHROPOIETIC PROTOPORPHYRIA (EPP)

- This chronic disease is caused by a deficiency in ferrochelatase.
- Protoporphyrin accumulates in erythrocytes, bone marrow, and plasma.
- Patients are photosensitive.

ACUTE INTERMITTENT PORPHYRIA (AIP)

- This acute disease is caused by a deficiency in hydroxymethylbilane synthase[2].
- Porphobilinogen and ALA accumulate in the urine.
- Urine darkens on exposure to light and air.
- Patients are not photosensitive.

VARIEGATE PORPHYRIA (VP)

- This acute disease is caused by a deficiency in protoporphyrinogen oxidase.
- Protoporphyrinogen IX and other intermediates prior to the block accumulate in the urine.
- Patients are photosensitive.

HEREDITARY COPROPORPHYRIA (HCP)

- This acute disease is caused by a deficiency in coproporphyrinogen III oxidase.
- Coproporphyrinogen III and other intermediates prior to the block accumulate in the urine.
- Patients are photosensitive.

PORPHYRIA CUTANEA TARDA (PCT)

- This chronic disease is caused by a deficiency in uroporphyrinogen decarboxylase.
- Uroporphyrin accumulates in the urine.
- It is the most common porphyria.
- Patients are photosensitive.

CONGENITAL ERYTHROPOIETIC PORPHYRIA (CEP)

- This chronic disease is caused by a deficiency in uroporphyrinogen III synthase.
- Uroporphyrinogen I and coproporphyrinogen I accumulate in the urine.
- Patients are photosensitive.

Heme
Fe^{2+}
Protoporphyrin IX
Protoporphyrinogen IX
Succinyl coenzyme A + Glycine
δ-Aminolevulinic acid
Coproporphyrinogen III
MITOCHONDRIA
CYTOSOL
δ-Aminolevulinic acid (ALA)
Coproporphyrinogen III
Spontaneous
Coproporphyrin III
Porphobilinogen
Hydroxymethylbilane
Uroporphyrinogen III
Spontaneous
Uroporphyrin III
Spontaneous
Uroporphyrinogen I
Uroporphyrin I
Spontaneous
Coproporphyrinogen I
Coproporphyrin I
KEY:
Hepatic porphyria
Erythropoietic porphyria

Figure 21.8

Summary of heme synthesis. [1]Also referred to as porphobilinogen synthase. [2]Also referred to as porphobilinogen deaminase. [Note: Deficiencies in ALA synthase-1 (ALAS1) are unknown. Deficiencies in ALAS2 result in an anemia, not a porphyria.]

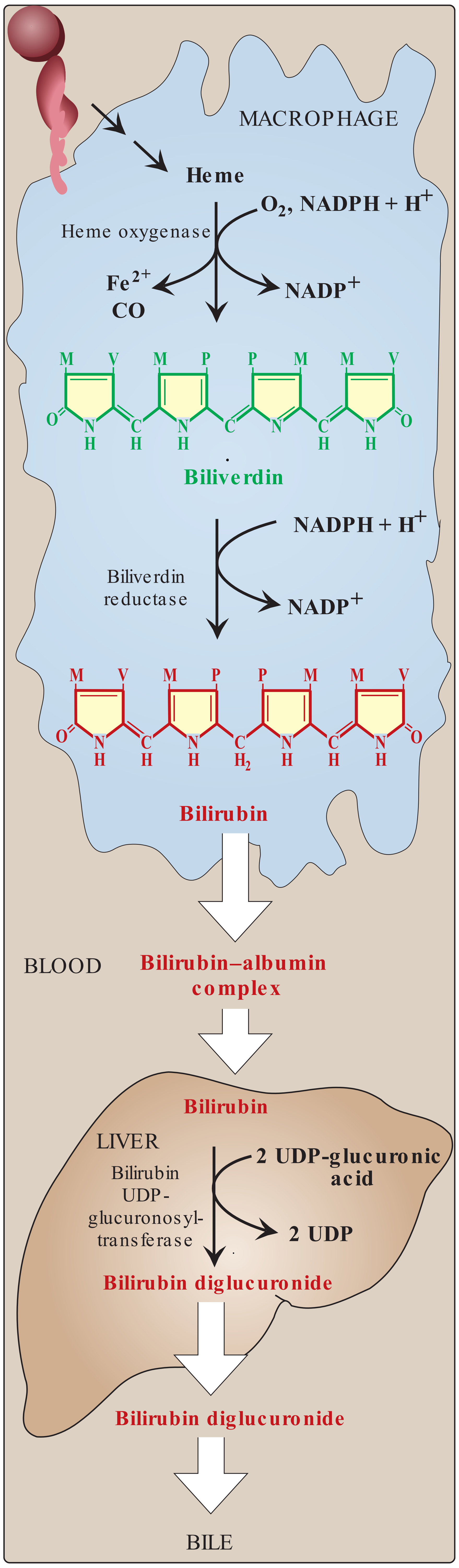


Figure 21.9
Formation of bilirubin from heme and its conversion to bilirubin diglucuronide. UDP = uridine diphosphate; CO = carbon monoxide; NADP(H) = nicotinamide adenine dinucleotide phosphate.

by intravenous injection of hemin and glucose, which decreases the synthesis of ALAS1. Protection from sunlight, ingestion of β-carotene (a free-radical scavenger), and phlebotomy are helpful in porphyrias with photosensitivity.

D. Degradation of heme

After approximately 120 days in the circulation, red blood cells are taken up and degraded by the reticuloendothelial system, particularly in the liver and spleen (Figure 21.9). Approximately 85% of heme destined for degradation comes from senescent RBCs. The remainder is from the degradation of heme proteins other than hemoglobin.

1. **Formation of bilirubin:** The first step in the degradation of heme is catalyzed by the microsomal heme oxygenase system of the reticuloendothelial cells. In the presence of nicotinamide adenine dinucleotide phosphate and O_2, the enzyme catalyzes three successive oxygenations that result in opening of the porphyrin ring (converting cyclic heme to linear biliverdin), production of carbon monoxide (CO), and release of Fe^{2+} (see Figure 21.9). [Note: The CO has biologic function, acting as a signaling molecule and anti-inflammatory.] Biliverdin, a green pigment, is reduced, forming the red-orange bilirubin. Bilirubin and its derivatives are collectively termed bile pigments. [Note: The changing colors of a bruise reflect the varying pattern of intermediates that occurs during heme degradation.]

> Bilirubin, unique to mammals, appears to function at low levels as an antioxidant. In this role, it is oxidized to biliverdin, which is then reduced by biliverdin reductase, regenerating bilirubin.

2. **Uptake of bilirubin by the liver:** Bilirubin is only slightly soluble in plasma and, therefore, is transported to the liver by binding noncovalently to albumin. [Note: Certain anionic drugs, such as salicylates and sulfonamides, can displace bilirubin from albumin, permitting bilirubin to enter the central nervous system (CNS). This causes the potential for neural damage in infants (see p. 285).] Bilirubin dissociates from the carrier albumin molecule; enters a hepatocyte via facilitated diffusion; and binds to intracellular proteins, particularly the protein ligandin.

3. **Formation of bilirubin diglucuronide:** In the hepatocyte, the solubility of bilirubin is increased by the addition of two molecules of glucuronic acid, producing bilirubin diglucuronide. [Note: This process is referred to as conjugation.] The reaction is catalyzed by microsomal bilirubin UDP-glucuronosyltransferase (bilirubin UGT) using uridine diphosphate (UDP)-glucuronic acid as the glucuronate donor. [Note: Varying degrees of deficiency of bilirubin UGT result in Crigler-Najjar I and II and Gilbert syndrome, with Crigler-Najjar I being the most severe deficiency.]

4. **Secretion of bilirubin into bile:** Bilirubin diglucuronide (conjugated bilirubin [CB]) is actively transported against a concentration gradient into the bile canaliculi and then into the bile. This

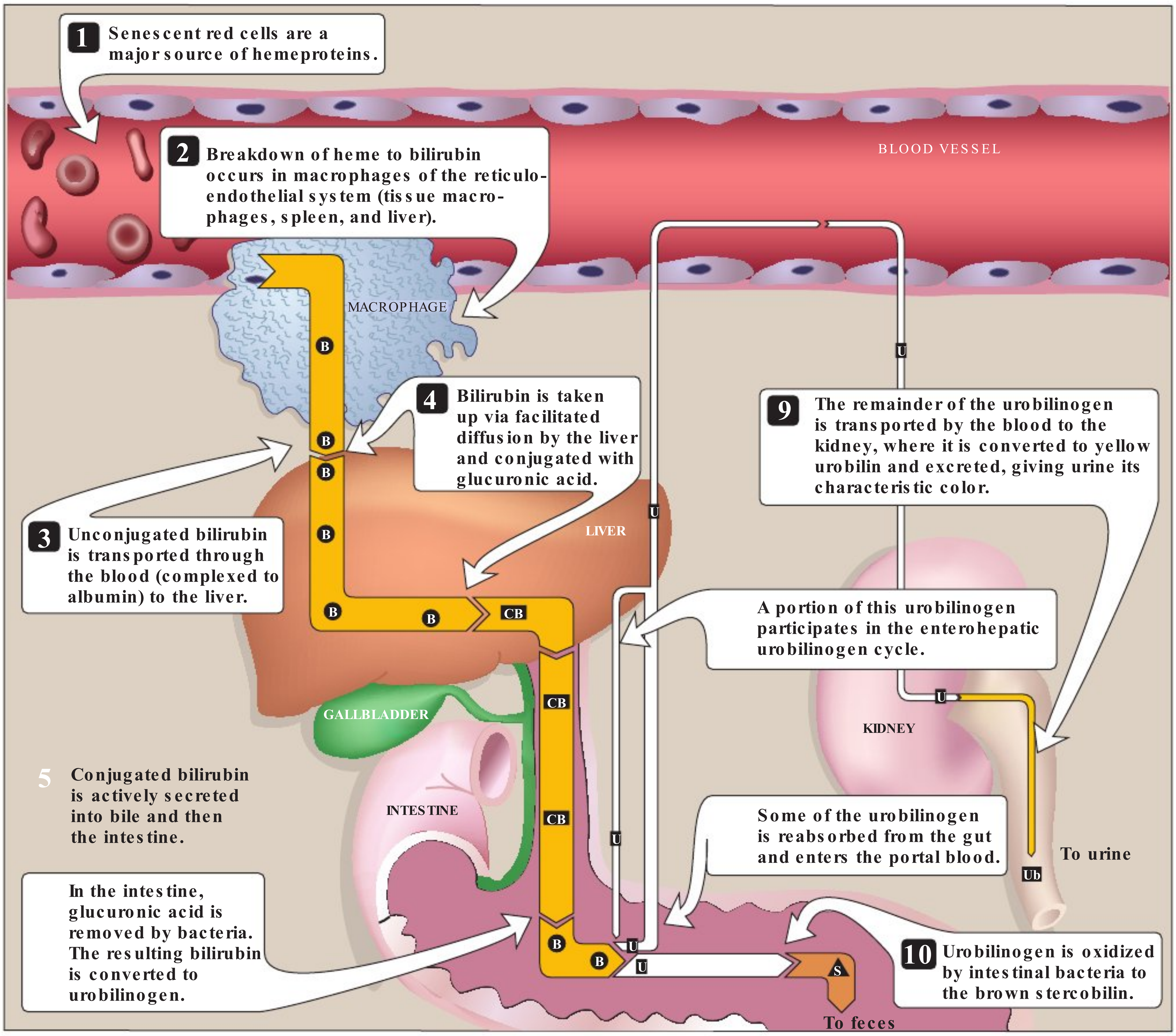

1 Senescent red cells are a major source of hemeproteins.
2 Breakdown of heme to bilirubin occurs in macrophages of the reticuloendothelial system (tissue macrophages, spleen, and liver).
BLOOD VESSEL
MACROPHAGE
B
B
B
B
B
B
CB
CB
CB
U
U
U
U
U
U
Ub
S
4 Bilirubin is taken up via facilitated diffusion by the liver and conjugated with glucuronic acid.
9 The remainder of the urobilinogen is transported by the blood to the kidney, where it is converted to yellow urobilin and excreted, giving urine its characteristic color.
3 Unconjugated bilirubin is transported through the blood (complexed to albumin) to the liver.
LIVER
A portion of this urobilinogen participates in the enterohepatic urobilinogen cycle.
GALLBLADDER
KIDNEY
5 Conjugated bilirubin is actively secreted into bile and then the intestine.
INTESTINE
Some of the urobilinogen is reabsorbed from the gut and enters the portal blood.
To urine
In the intestine, glucuronic acid is removed by bacteria. The resulting bilirubin is converted to urobilinogen.
10 Urobilinogen is oxidized by intestinal bacteria to the brown stercobilin.
To feces

Figure 21.10
Catabolism of heme Ⓑ = bilirubin; CB = conjugated bilirubin; U = urobilinogen; Ub = urobilin; S = stercobilin.

energy-dependent, rate-limiting step is susceptible to impairment in liver disease. [Note: A rare deficiency in the protein required for transport of CB out of the liver results in Dubin-Johnson syndrome.] Unconjugated bilirubin (UCB) is normally not secreted into bile.

5. **Formation of urobilins in the intestine:** Bilirubin diglucuronide is hydrolyzed and reduced by bacteria in the gut to yield urobilinogen, a colorless compound. Most of the urobilinogen is oxidized by intestinal bacteria to stercobilin, which gives feces the characteristic brown color. However, some of the urobilinogen is reabsorbed from the gut and enters the portal blood. A portion of this urobilinogen participates in the enterohepatic urobilinogen cycle in which it is taken up by the liver and then resecreted into the bile. The remainder of the urobilinogen is transported by the

blood to the kidney, where it is converted to yellow urobilin and excreted, giving urine its characteristic color. The metabolism of bilirubin is summarized in Figure 21.10.

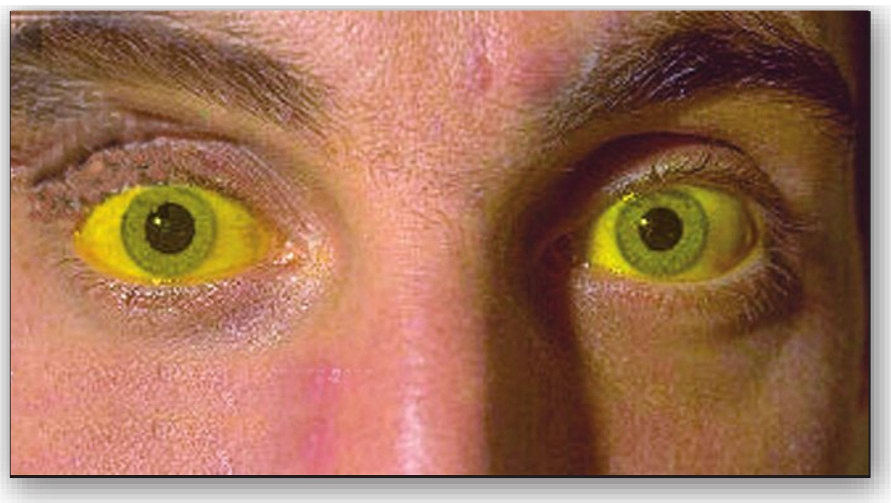


Figure 21.11
Jaundiced patient, with the sclerae of his eyes appearing yellow.

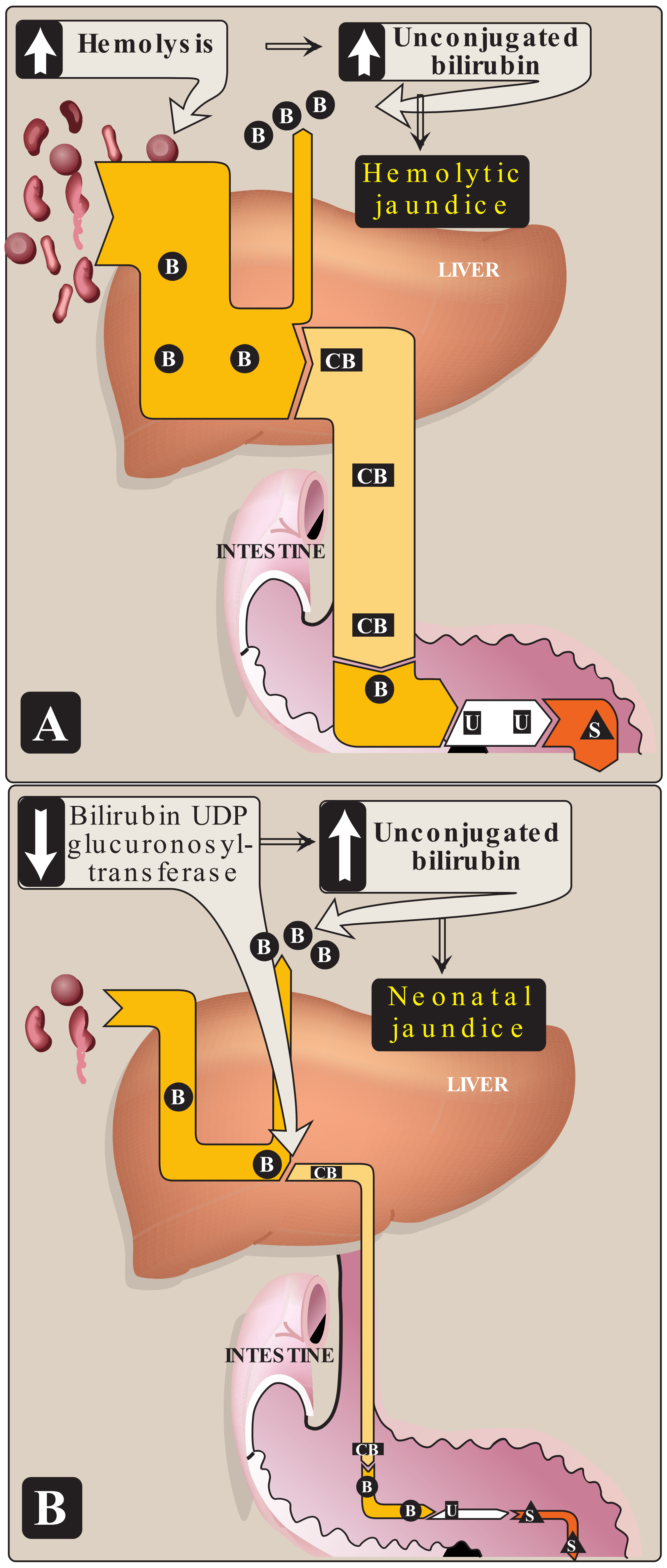


Figure 21.12
Alterations in the metabolism of heme.
A. Hemolytic jaundice. B. Neonatal jaundice.
CB = conjugated bilirubin; Ⓑ = bilirubin;
U = urobilinogen; S = stercobilin;
UDP = uridine diphosphate.

E. Jaundice

Jaundice (also called icterus) refers to the yellow color of skin, nail beds, and sclerae (whites of the eyes) caused by deposition of bilirubin, secondary to increased bilirubin levels in the blood (hyperbilirubinemia) as shown in Figure 21.11. Although not a disease, jaundice is usually a symptom of an underlying disorder. [Note: Blood bilirubin levels are normally about 1 mg/dl. Jaundice is seen at 2–3 mg/dl.]

1. **Types of jaundice:** Jaundice can be classified into three major types described below. However, in clinical practice, jaundice is often more complex than indicated in this simple classification. For example, the accumulation of bilirubin may be a result of defects at more than one step in its metabolism.

   a. **Hemolytic jaundice:** The liver has the capacity to conjugate and excrete over 3,000 mg of bilirubin per day, whereas the normal production of bilirubin is only 300 mg/day. This excess capacity allows the liver to respond to increased heme degradation with a corresponding increase in conjugation and secretion of bilirubin diglucuronide. However, extensive hemolysis (for example, in patients with sickle cell anemia or pyruvate kinase or glucose 6-phosphate dehydrogenase deficiency) may produce bilirubin faster than it can be conjugated. UCB levels in the blood become elevated, causing jaundice (Figure 21.12A). [Note: With hemolysis, more CB is made and excreted into the bile, the amount of urobilinogen entering the enterohepatic circulation is increased, and urinary urobilinogen is increased.]

   b. **Hepatocellular jaundice:** Damage to liver cells (for example, in patients with cirrhosis or hepatitis) can cause UCB levels in the blood to increase as a result of decreased conjugation. Urobilinogen is increased in the urine because hepatic damage decreases the enterohepatic circulation of this compound, allowing more to enter the blood, from which it is filtered into the urine. The urine consequently darkens, whereas stools may be a pale, clay color. Plasma levels of alanine and aspartate transaminases (AST and ALT, respectively; see p. 251) are elevated. [Note: If CB is made but is not efficiently secreted from the liver into bile (intrahepatic cholestasis), it can diffuse ("leak") into the blood, causing a conjugated hyperbilirubinemia.]

   c. **Obstructive jaundice:** In this instance, jaundice is not caused by overproduction of bilirubin or decreased conjugation but, instead, results from obstruction of the common bile duct (extrahepatic cholestasis). For example, the presence of a tumor or bile stones may block the duct, preventing passage of CB into the intestine. Patients with obstructive jaundice experience GI pain and nausea and produce stools that are a pale, clay color. The liver "regurgitates" CB into the blood (hyperbilirubinemia). The CB is eventually excreted in the urine (which darkens upon standing), and is referred to as "urinary bilirubin." Urinary urobilinogen is absent.

2. **Jaundice in newborns:** The majority of newborn infants (60% of full term and 80% of preterm) show a rise in UCB in the first postnatal week (and a transient, "physiologic" jaundice) because the activity of hepatic bilirubin UGT is low at birth (it reaches adult levels in about 4 weeks) as shown in Figures 21.12B and 21.13. Elevated UCB, in excess of the binding capacity of albumin (20–25 mg/dl), can diffuse into the basal ganglia, cause toxic encephalopathy (kernicterus) and a pathologic jaundice. Therefore, newborns with significantly elevated bilirubin levels are treated with blue fluorescent light (phototherapy), as shown in Figure 21.14, which converts bilirubin to more polar and, therefore, water-soluble isomers. These photoisomers can be excreted into the bile without conjugation to glucuronic acid. [Note: Because of solubility differences, only UCB crosses the blood brain barrier, and only CB appears in urine.]

3. **Determination of bilirubin concentration:** Bilirubin is commonly measured by the van den Bergh reaction, in which diazotized sulfanilic acid reacts with bilirubin to form red azodipyrroles that are measured colorimetrically. In aqueous solution, the water-soluble CB reacts rapidly with the reagent (within one minute), and is said to be "direct-reacting." The UCB, which is much less soluble in aqueous solution, reacts more slowly. However, when the reaction is carried out in methanol, both CB and UCB are soluble and react with the reagent, providing the total bilirubin value. The "indirect-reacting" bilirubin, which corresponds to the UCB, is obtained by subtracting the direct-reacting bilirubin from the total bilirubin. [Note: In normal plasma, only about 4% of the total bilirubin is conjugated or direct-reacting, because most is secreted into bile.]

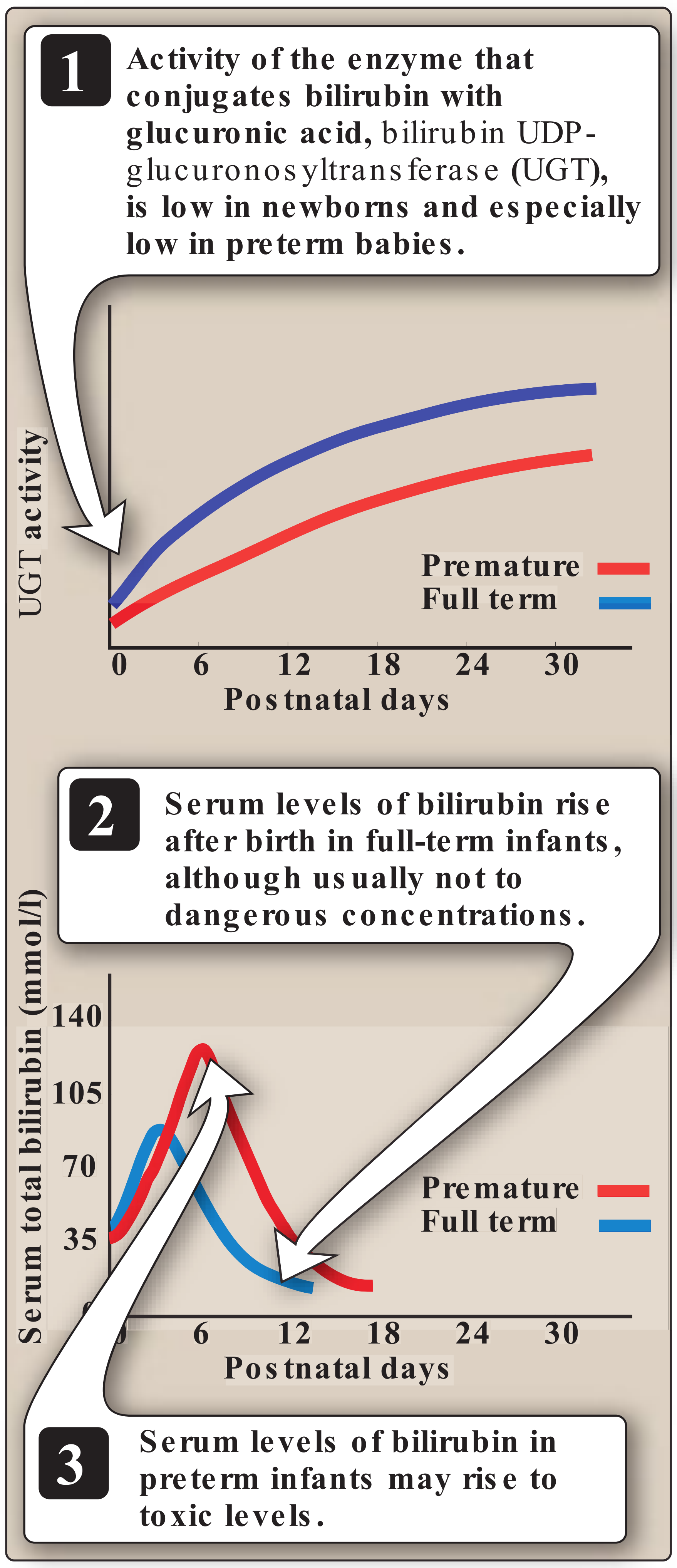


Figure 21.13
Neonatal jaundice. UDP = uridine diphosphate.

Figure 21.14
Phototherapy in neonatal jaudice.

III. OTHER NITROGEN-CONTAINING COMPOUNDS

A. Catecholamines

Dopamine, norepinephrine, and epinephrine are biologically active (biogenic) amines that are collectively termed catecholamines. Dopamine and norepinephrine are synthesized in the brain and function as neurotransmitters. Norepinephrine is also synthesized in the adrenal medulla, as is epinephrine.

1. **Function:** Outside the CNS, norepinephrine and its methylated derivative, epinephrine, are hormone regulators of carbohydrate and lipid metabolism. Norepinephrine and epinephrine are released from storage vesicles in the adrenal medulla in response to fright, exercise, cold, and low levels of blood glucose. They increase the degradation of glycogen and triacylglycerol as well as increase blood pressure and the output of the heart. These effects are part of a coordinated response to prepare the individual for stress, and are often called the "fight-or-flight" reactions.

2. **Synthesis:** The catecholamines are synthesized from tyrosine, as shown in Figure 21.15. Tyrosine is first hydroxylated by tyrosine hydroxylase to form 3,4-dihydroxyphenylalanine (DOPA) in a reaction analogous to that described for the hydroxylation of phenylalanine (see p. 270). The tetrahydrobiopterin (BH_4)-requiring

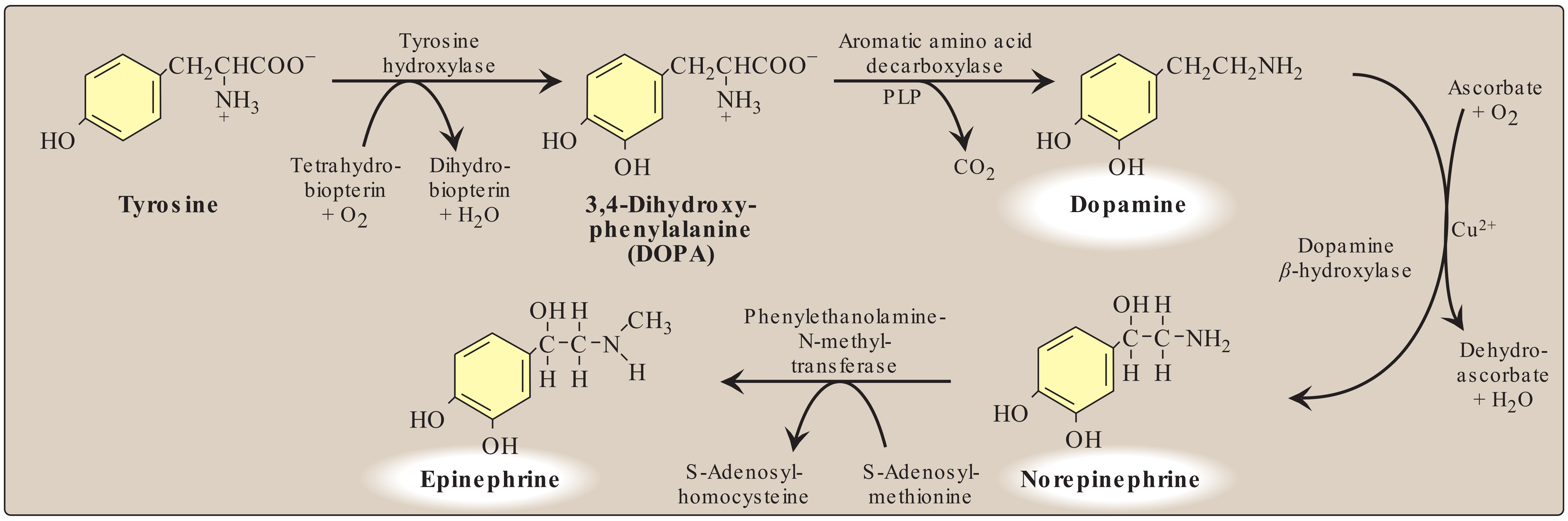

Tyrosine
HO
CH_2CHCOO^-
$\overset{+}{NH_3}$
Tyrosine hydroxylase
Tetrahydro-biopterin + O_2
Dihydro-biopterin + H_2O
3,4-Dihydroxy-phenylalanine (DOPA)
OH
Aromatic amino acid decarboxylase
PLP
CO_2
Dopamine
$CH_2CH_2NH_2$
Ascorbate + O_2
Cu^{2+}
Dopamine β-hydroxylase
Dehydro-ascorbate + H_2O
Norepinephrine
NH_2
Phenylethanolamine-N-methyl-transferase
S-Adenosyl-homocysteine
S-Adenosyl-methionine
Epinephrine
CH_3

Figure 21.15
Synthesis of catecholamines. PLP = pyridoxal phosphate; Cu^{2+} = copper.

enzyme is abundant in the CNS, the sympathetic ganglia, and the adrenal medulla and is the rate-limiting step of the pathway. DOPA is decarboxylated in a reaction requiring PLP (see p. 378) to form dopamine, which is hydroxylated by dopamine *β*-hydroxylase to yield norepinephrine in a reaction that requires ascorbate (vitamin C) and copper. Epinephrine is formed from norepinephrine by an N-methylation reaction using S-adenosylmethionine (SAM) as the methyl donor (see p. 264).

Parkinson disease, a neurodegenerative movement disorder, is due to insufficient dopamine production as a result of the idiopathic loss of dopamine-producing cells in the brain. Administration of L-DOPA (levodopa) is the most common treatment. Dopamine cannot cross the blood brain barrier.

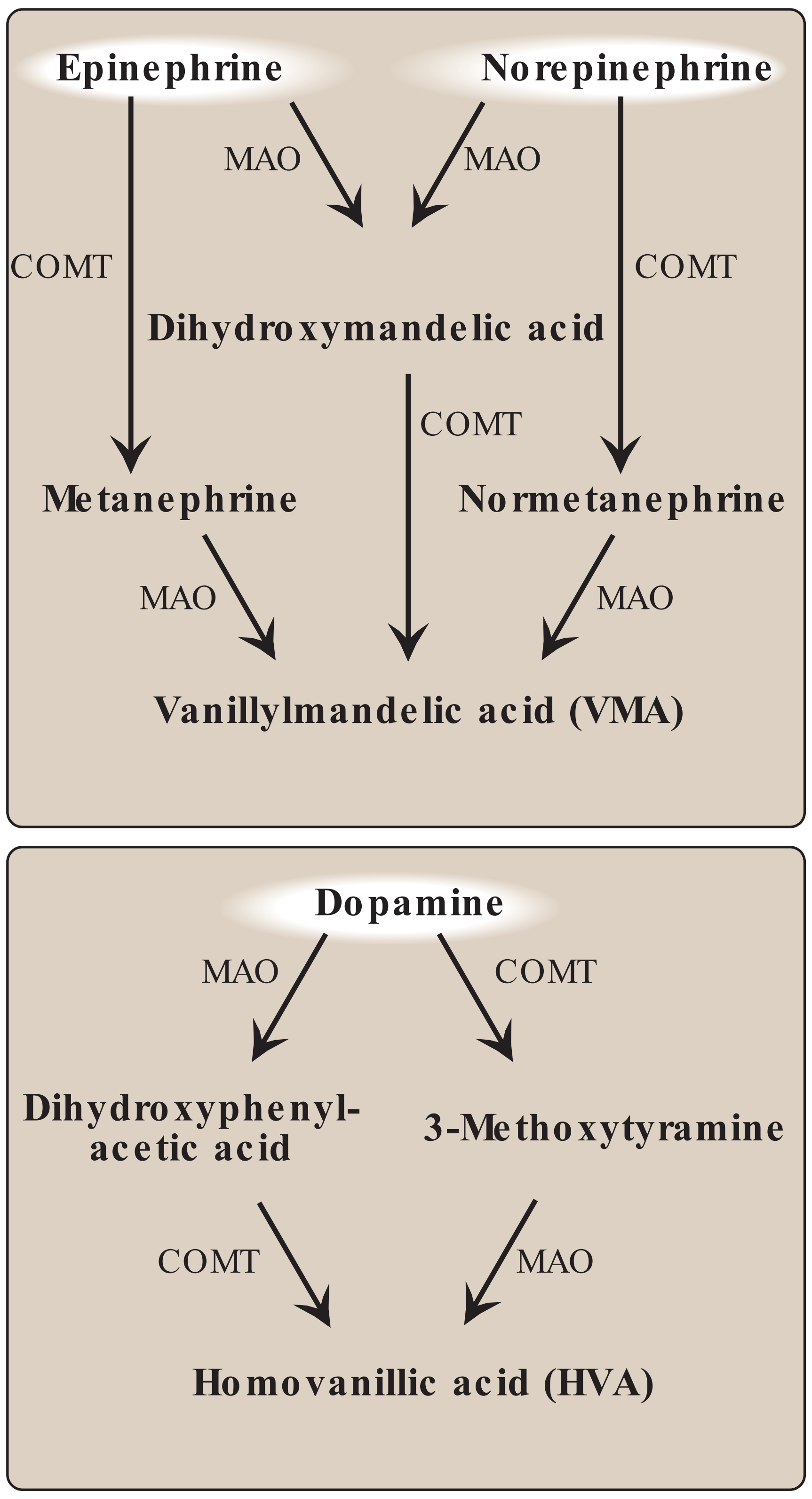

Epinephrine
Norepinephrine
MAO
MAO
COMT
COMT
Dihydroxymandelic acid
COMT
Metanephrine
Normetanephrine
MAO
MAO
Vanillylmandelic acid (VMA)
Dopamine
MAO
COMT
Dihydroxyphenyl-acetic acid
3-Methoxytyramine
COMT
MAO
Homovanillic acid (HVA)

Figure 21.16
Metabolism of the catecholamines by catechol-O-methyltranferase (COMT) and monoamine oxidase (MAO).

3. **Degradation:** The catecholamines are inactivated by oxidative deamination catalyzed by monoamine oxidase (MAO) and by O-methylation carried out by catechol-O-methyltransferase (COMT) using SAM as the methyl donor (Figure 21.16). The two reactions can occur in either order. The aldehyde products of the MAO reaction are oxidized to the corresponding acids. The metabolic products of these reactions are excreted in the urine as vanillylmandelic acid (VMA) from epinephrine and norepinephrine and homovanillic acid from dopamine. [Note: VMA is increased with pheochromocytomas, rare tumors of the adrenal gland characterized by excessive production of catecholamines.]

4. **Monoamine oxidase inhibitors:** MAO is found in neural and other tissues, such as the intestine and liver. In the neuron, this enzyme oxidatively deaminates and inactivates any excess neurotransmitter molecules (norepinephrine, dopamine, or serotonin) that may leak out of synaptic vesicles when the neuron is at rest. MAO inhibitors may irreversibly or reversibly inactivate the enzyme, permitting neurotransmitter molecules to escape

degradation and, therefore, to both accumulate within the presynaptic neuron and to leak into the synaptic space. This causes activation of norepinephrine and serotonin receptors and may be responsible for the antidepressant action of these drugs.

B. Histamine

Histamine is a chemical messenger that mediates a wide range of cellular responses, including allergic and inflammatory reactions and gastric acid secretion. A powerful vasodilator, histamine is formed by decarboxylation of histidine in a reaction requiring PLP (Figure 21.17). It is secreted by mast cells as a result of allergic reactions or trauma. Histamine has no clinical applications, but agents that interfere with the action of histamine have important therapeutic applications.

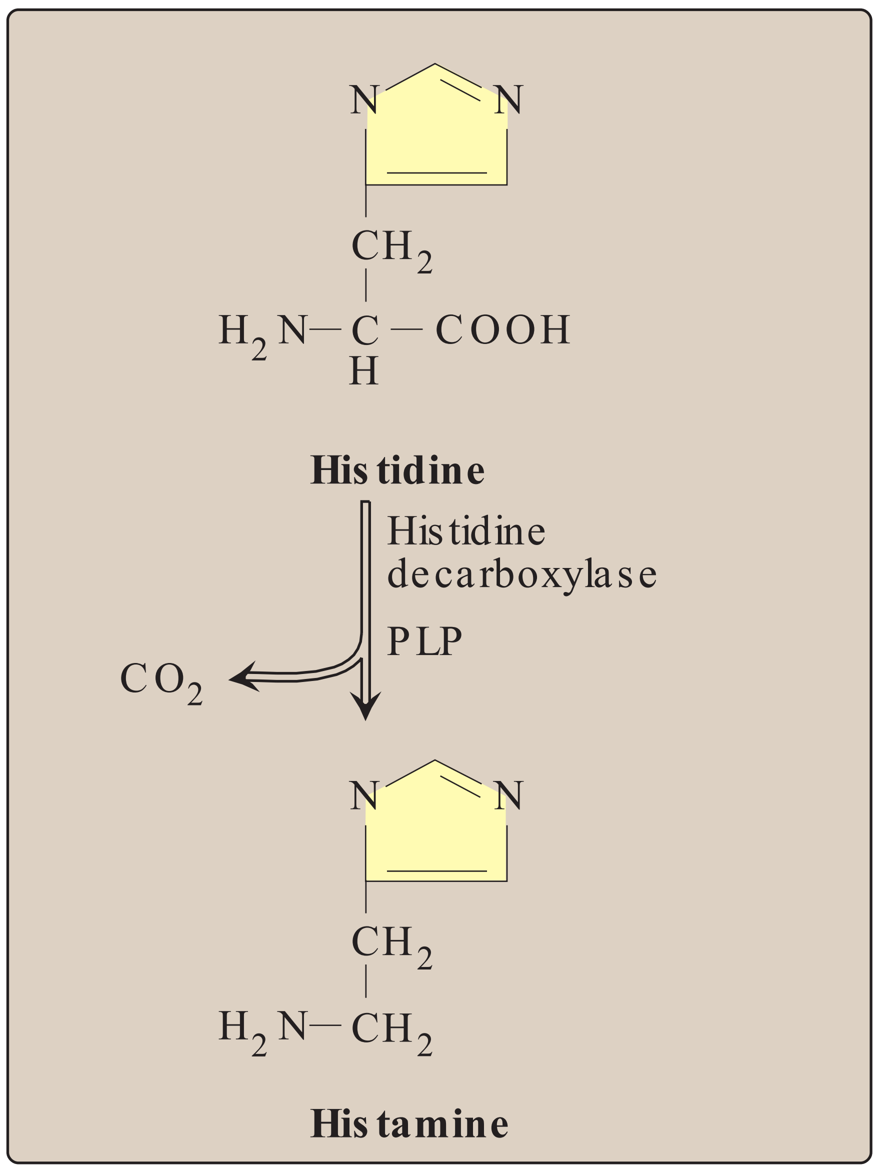


Figure 21.17
Biosynthesis of histamine. PLP = pyridoxal phosphate.

C. Serotonin

Serotonin, also called 5-hydroxytryptamine (5-HT), is synthesized and stored at several sites in the body (Figure 21.18). The largest amount by far is found in the intestinal mucosa. Smaller amounts occur in the CNS, where it functions as a neurotransmitter, and in platelets. Serotonin is synthesized from tryptophan, which is hydroxylated in a BH_4-requiring reaction analogous to that catalyzed by phenylalanine hydroxylase. The product, 5-hydroxytryptophan, is decarboxylated to serotonin, which is degraded by MAO. Serotonin has multiple physiologic roles including pain perception, regulation of sleep, appetite, temperature, blood pressure, cognitive functions, and mood (causes a feeling of well-being). [Note: Selective serotonin reuptake inhibitors (SSRIs) maintain serotonin levels, thereby functioning as antidepressants.]

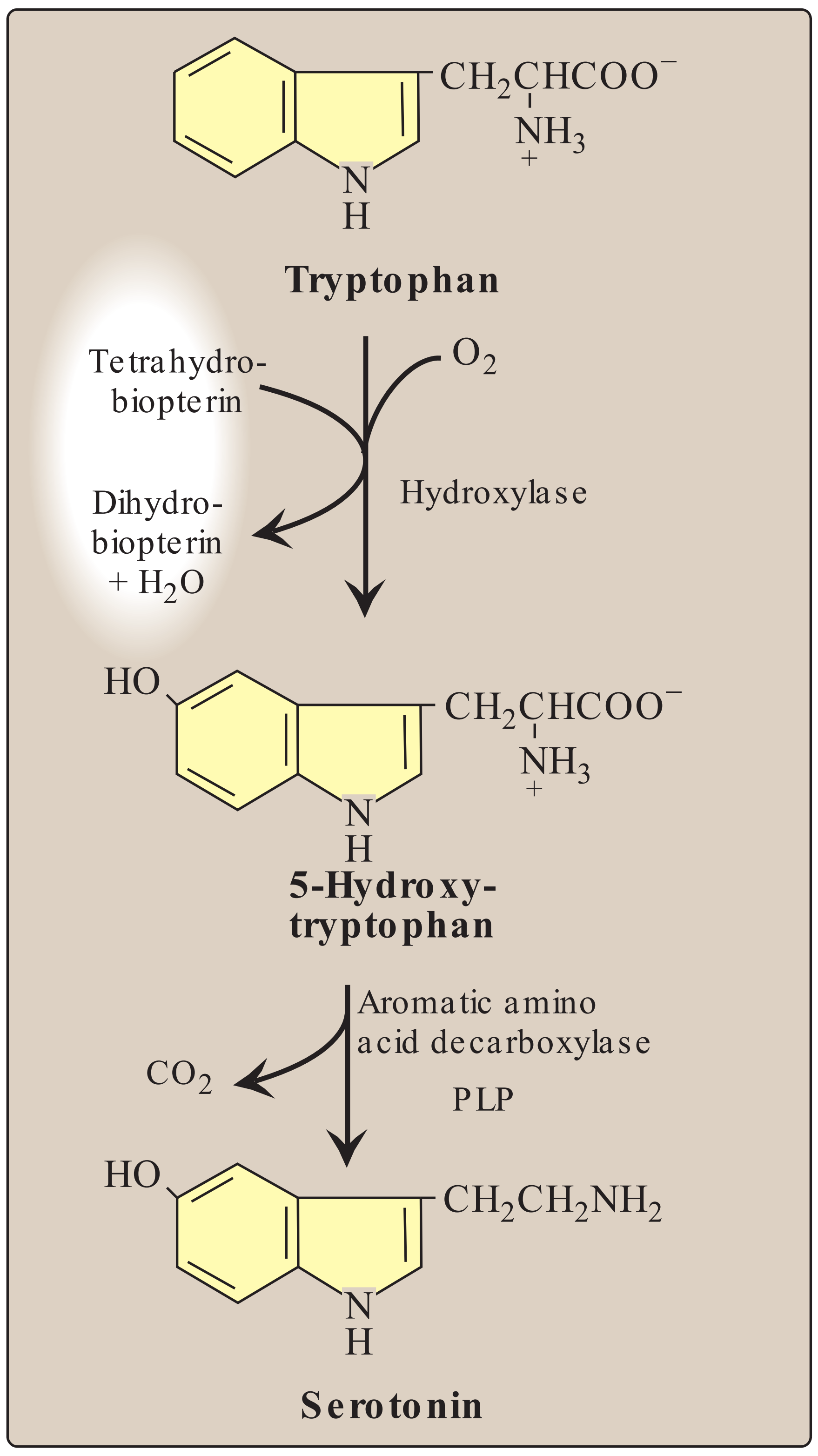


Figure 21.18
Synthesis of serotonin. [Note: Serotonin is converted to melatonin in the pineal gland.] PLP = pyridoxal phosphate.

D. Creatine

Creatine phosphate (also called phosphocreatine), the phosphorylated derivative of creatine found in muscle, is a high-energy compound that provides a small but rapidly mobilized reserve of high-energy phosphates that can be reversibly transferred to adenosine diphosphate (Figure 21.19) to maintain the intracellular level of adenosine triphosphate (ATP) during the first few minutes of intense muscular contraction. [Note: The amount of creatine phosphate in the body is proportional to the muscle mass.]

1. **Synthesis:** Creatine is synthesized in liver and kidney tissue from glycine and the guanidino group of arginine, plus a methyl group from SAM (see Figure 21.19). Animal products are dietary sources. Creatine is reversibly phosphorylated to creatine phosphate by creatine kinase, using ATP as the phosphate donor. [Note: The presence of creatine kinase (MB isozyme) in the plasma is indicative of heart damage and is used in the diagnosis of myocardial infarction (see p. 65).]

2. **Degradation:** Creatine and creatine phosphate spontaneously cyclize at a slow but constant rate to form creatinine, which is excreted in the urine. The amount excreted is proportional to the total creatine phosphate content of the body and, therefore, can be used to estimate muscle mass. When muscle mass decreases for any reason (for example, from paralysis or muscular dystrophy),

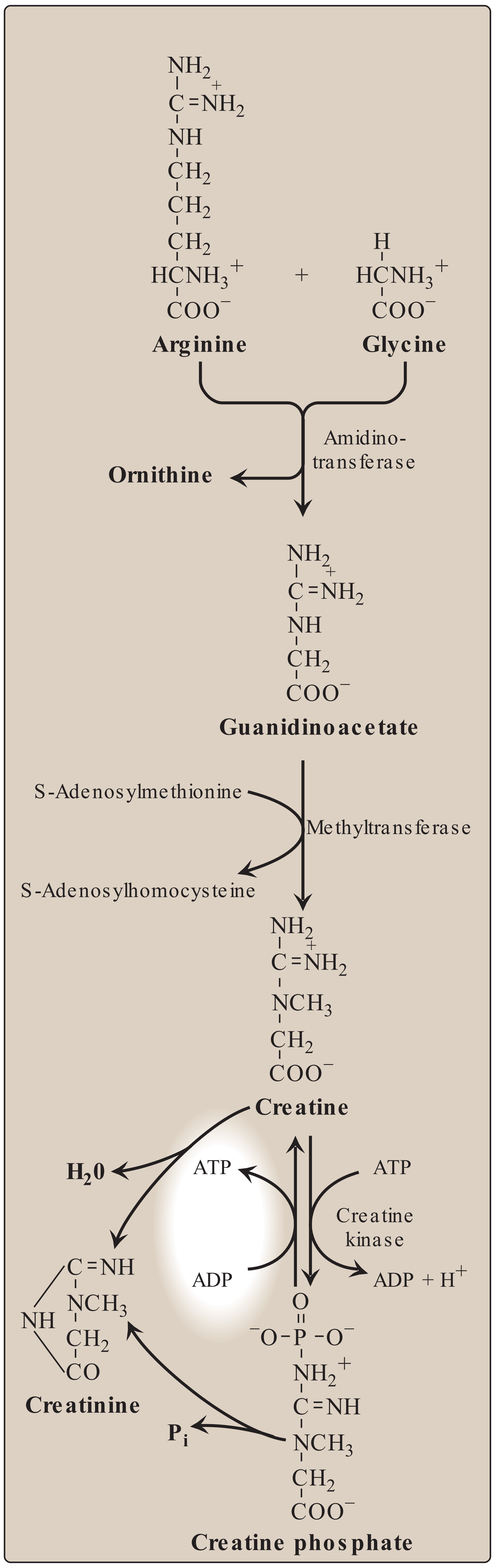


Figure 21.19
Synthesis of creatine. ADP = adenosine diphosphate; P_i = inorganic phosphate.

the creatinine content of the urine falls. In addition, any rise in blood creatinine is a sensitive indicator of kidney malfunction, because creatinine normally is rapidly removed from the blood and excreted. A typical adult male excretes about 1–2 g of creatinine per day.

E. Melanin

Melanin is a pigment that occurs in several tissues, particularly the eye, hair, and skin. It is synthesized from tyrosine in melanocytes (pigment-forming cells) of the epidermis. It functions to protect underlying cells from the harmful effects of sunlight. [Note: A defect in melanin production results in oculocutaneous albinism, the most common type being due to defects in copper-containing tyrosinase (see p. 273).]

IV. CHAPTER SUMMARY

Amino acids are precursors of many nitrogen-containing compounds including **porphyrins**, which, in combination with ferrous (Fe^{2+}) iron, form **heme** (Figure 21.20). The major sites of **heme biosynthesis** are the **liver**, which synthesizes a number of heme proteins (particularly cytochrome P450 enzymes), and the **erythrocyte-producing cells** of the bone marrow, which are active in hemoglobin synthesis. In the liver, the rate of heme synthesis is highly variable, responding to alterations in the cellular heme pool caused by fluctuating demands for hemeproteins. In contrast, heme synthesis in erythroid cells is relatively constant and is matched to the rate of globin synthesis. **Porphyrin synthesis** start with **glycine** and **succinyl coenzyme A**. The **committed step** in heme synthesis is the formation of d-aminolevulinic acid (ALA). This reaction is catalyzed by ALA synthase-1 (ALAS1) in liver (inhibited by **hemin**, the oxidized form of heme that accumulates in the cell when heme is being underutilized) and ALAS2 in erythroid tissues (regulated by **iron**). **Porphyrias** are caused by inherited (primarily **autosomal-dominant**) or acquired defects in heme synthesis, resulting in the accumulation and increased excretion of porphyrins or porphyrin precursors. Enzymic defects early in the pathway cause **abdominal pain** and **neuropsychiatric symptoms**, whereas later defects cause **photosensitivity**. **Degradation** of hemeproteins occurs in the **reticuloendothelial system**, particularly in the **liver** and **spleen**. The first step in the degradation of heme is the production by heme oxygenase of the green pigment **biliverdin**, which is subsequently reduced to **bilirubin**. Bilirubin is transported to the liver, where its solubility is increased by the addition of two molecules of **glucuronic acid**. Bilirubin diglucuronide is transported into the **bile canaliculi**, where it is first hydrolyzed and reduced by bacteria in the gut to yield **urobilinogen**, then oxidized by intestinal bacteria to **stercobilin**. **Jaundice** refers to the yellow color of the skin and sclerae that is caused by deposition of bilirubin, secondary to increased bilirubin levels in the blood. Three commonly encountered type of jaundice are **hemolytic jaundice**, **obstructive jaundice**, and **hepatocellular jaundice**. Other important N-containing compounds derived from amino acids include the **catecholamines** (dopamine, norepinephrine, and epinephrine), **creatine**, **histamine**, **serotonin**, and **melanin**.

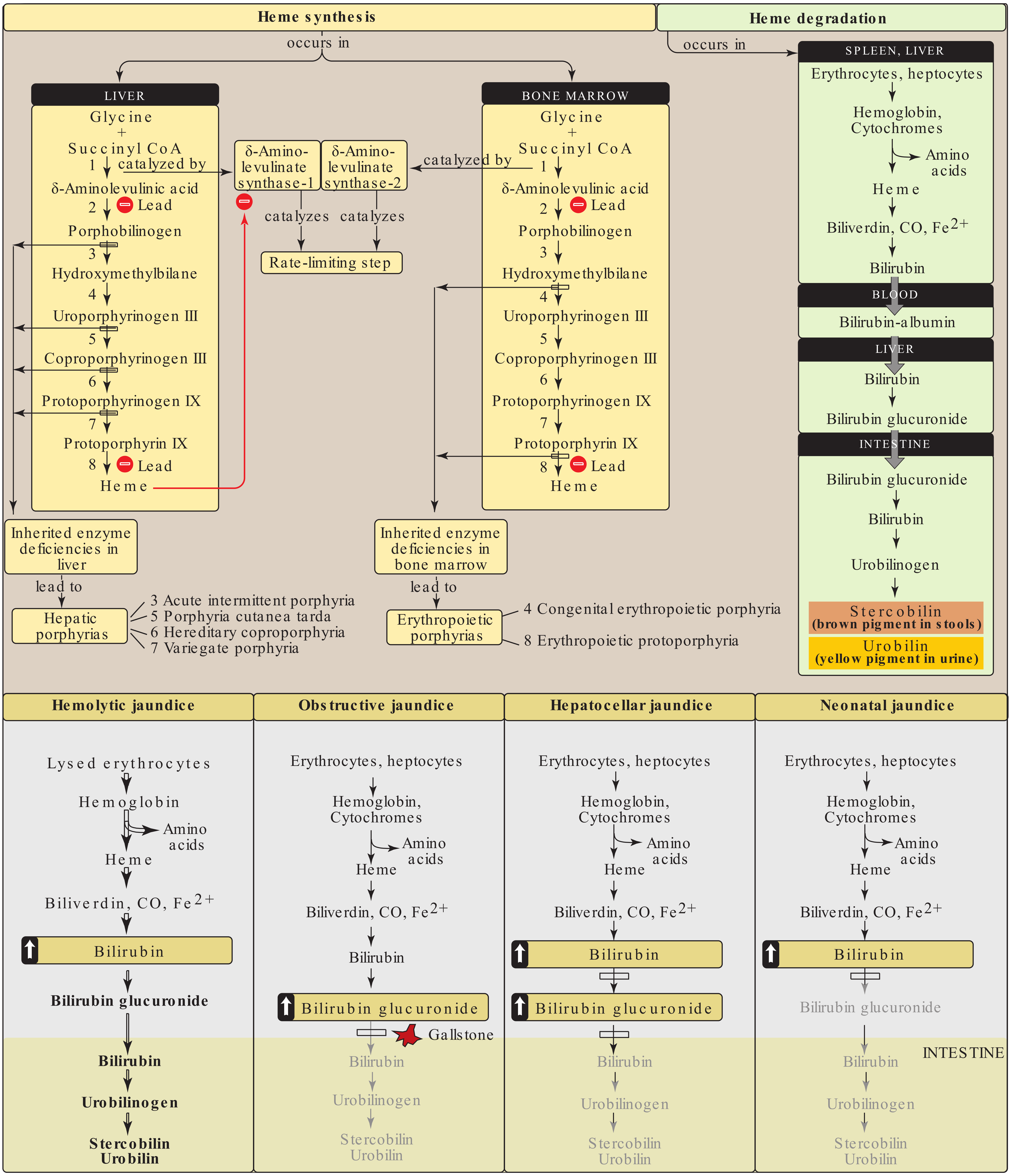

Heme synthesis
occurs in
LIVER
Glycine
+
Succinyl CoA
1 catalyzed by
δ-Aminolevulinic acid
2 Lead
Porphobilinogen
3
Hydroxymethylbilane
4
Uroporphyrinogen III
5
Coproporphyrinogen III
6
Protoporphyrinogen IX
7
Protoporphyrin IX
8 Lead
Heme
δ-Aminolevulinate synthase-1
δ-Aminolevulinate synthase-2
catalyzes
catalyzes
Rate-limiting step
catalyzed by
BONE MARROW
Glycine
+
Succinyl CoA
1
δ-Aminolevulinic acid
2 Lead
Porphobilinogen
3
Hydroxymethylbilane
4
Uroporphyrinogen III
5
Coproporphyrinogen III
6
Protoporphyrinogen IX
7
Protoporphyrin IX
8 Lead
Heme
Inherited enzyme deficiencies in liver
lead to
Hepatic porphyrias
3 Acute intermittent porphyria
5 Porphyria cutanea tarda
6 Hereditary coproporphyria
7 Variegate porphyria
Inherited enzyme deficiencies in bone marrow
lead to
Erythropoietic porphyrias
4 Congenital erythropoietic porphyria
8 Erythropoietic protoporphyria
Heme degradation
occurs in
SPLEEN, LIVER
Erythrocytes, heptocytes
Hemoglobin, Cytochromes
Amino acids
Heme
Biliverdin, CO, Fe2+
Bilirubin
BLOOD
Bilirubin-albumin
LIVER
Bilirubin
Bilirubin glucuronide
INTESTINE
Bilirubin glucuronide
Bilirubin
Urobilinogen
Stercobilin (brown pigment in stools)
Urobilin (yellow pigment in urine)
Hemolytic jaundice
Lysed erythrocytes
Hemoglobin
Amino acids
Heme
Biliverdin, CO, Fe2+
Bilirubin
Bilirubin glucuronide
Bilirubin
Urobilinogen
Stercobilin
Urobilin
Obstructive jaundice
Erythrocytes, heptocytes
Hemoglobin, Cytochromes
Amino acids
Heme
Biliverdin, CO, Fe2+
Bilirubin
Bilirubin glucuronide
Gallstone
Bilirubin
Urobilinogen
Stercobilin
Urobilin
Hepatocellular jaundice
Erythrocytes, heptocytes
Hemoglobin, Cytochromes
Amino acids
Heme
Biliverdin, CO, Fe2+
Bilirubin
Bilirubin glucuronide
Bilirubin
Urobilinogen
Stercobilin
Urobilin
Neonatal jaundice
Erythrocytes, heptocytes
Hemoglobin, Cytochromes
Amino acids
Heme
Biliverdin, CO, Fe2+
Bilirubin
Bilirubin glucuronide
INTESTINE
Bilirubin
Urobilinogen
Stercobilin
Urobilin

Figure 21.20
Key concept map for heme metabolism. ▭ = Block in the pathway. [Note: Hepatocellular jaundice can be caused by decreased conjugation of bilirubin or decreased secretion of conjugated bilirubin into bile.] CoA = coenzyme A; CO = carbon monoxide.

Nucleotide Metabolism

22

I. OVERVIEW

Ribonucleoside and deoxyribonucleoside phosphates (nucleotides) are essential for all cells. Without them, neither ribonucleic acid (RNA) nor deoxyribonucleic acid (DNA) can be produced, and, therefore, proteins cannot be synthesized or cells proliferate. Nucleotides also serve as carriers of activated intermediates in the synthesis of some carbohydrates, lipids, and conjugated proteins (for example, uridine diphosphate [UDP]-glucose and cytidine diphosphate [CDP]-choline) and are structural components of several essential coenzymes, such as coenzyme A, flavin adenine dinucleotide (FAD[H_2]), nicotinamide adenine dinucleotide (NAD[H]), and nicotinamide adenine dinucleotide phosphate (NADP[H]). Nucleotides, such as cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), serve as second messengers in signal transduction pathways. In addition, nucleotides play an important role as "energy currency" in the cell. Finally, nucleotides are important regulatory compounds for many of the pathways of intermediary metabolism, inhibiting or activating key enzymes. The purine and pyrimidine bases found in nucleotides can be synthesized de novo or can be obtained through salvage pathways that allow the reuse of the preformed bases resulting from normal cell turnover. [Note: Little of the purines and pyrimidines supplied by diet are utilized and are degraded instead.]

II. NUCLEOTIDE STRUCTURE

Nucleotides are composed of a nitrogenous base; a pentose monosaccharide; and one, two, or three phosphate groups. The nitrogen-containing bases belong to two families of compounds: the purines and the pyrimidines.

A. Purine and pyrimidine structures

Both DNA and RNA contain the same purine bases: adenine (A) and guanine (G). Both DNA and RNA contain the pyrimidine

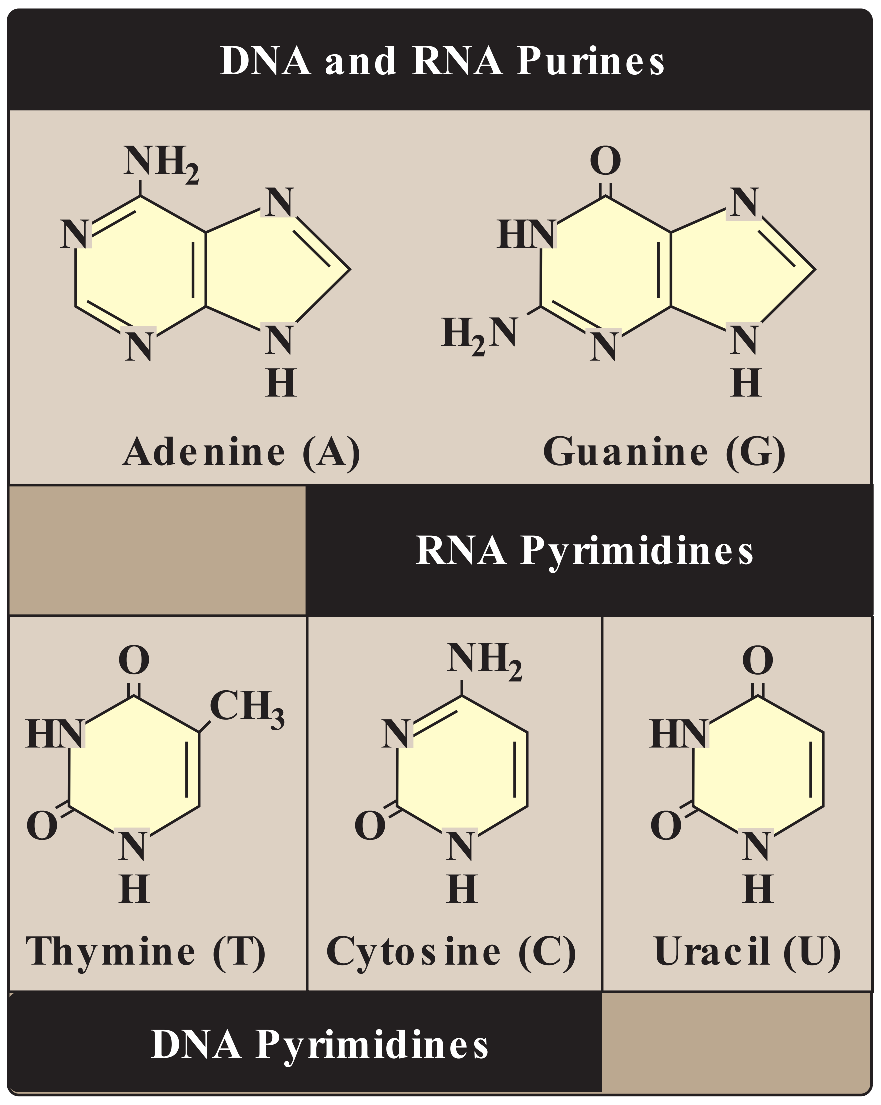


Figure 22.1
Purines and pyrimidines commonly found in DNA and RNA.

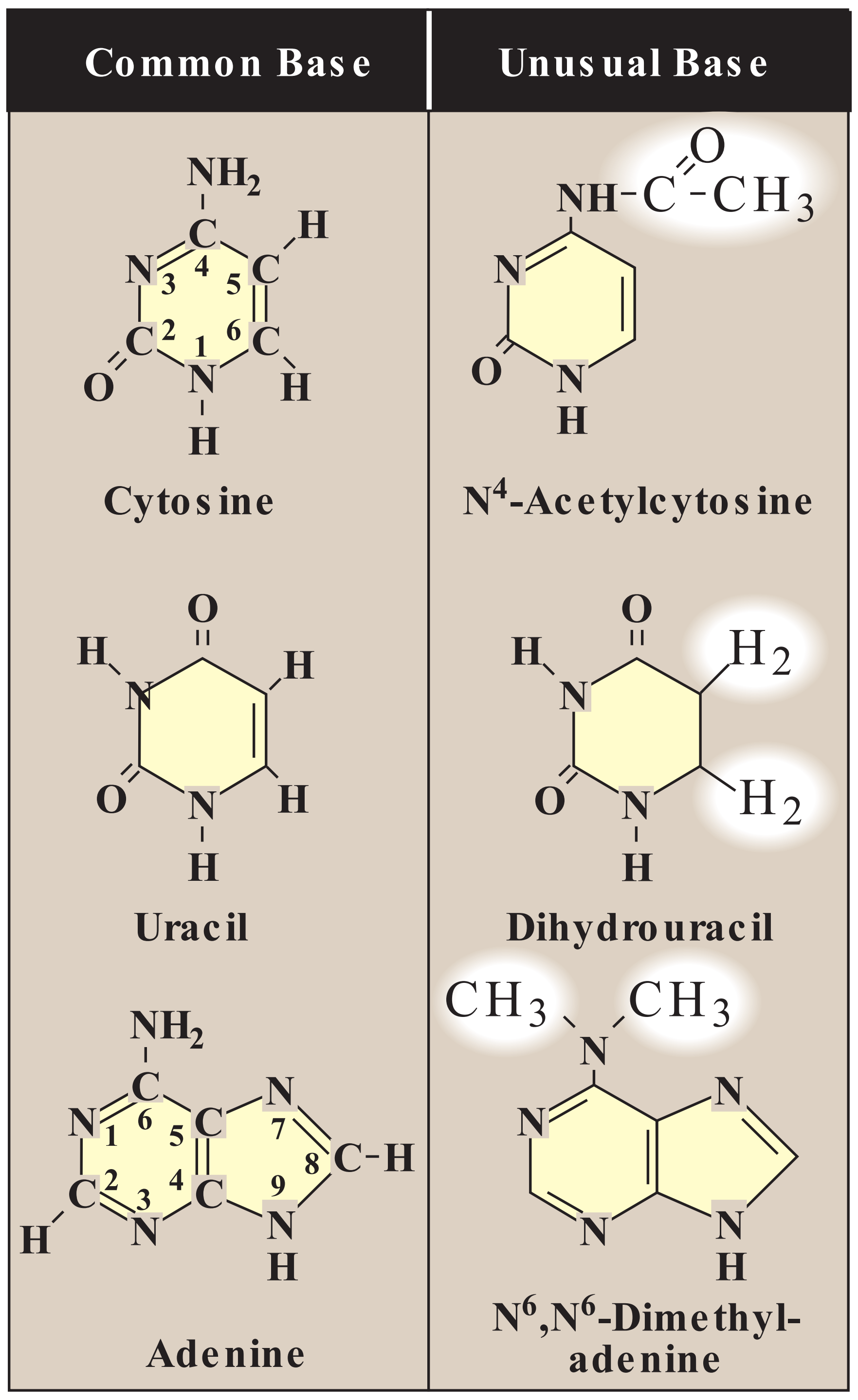


Figure 22.2
Examples of unusual bases.

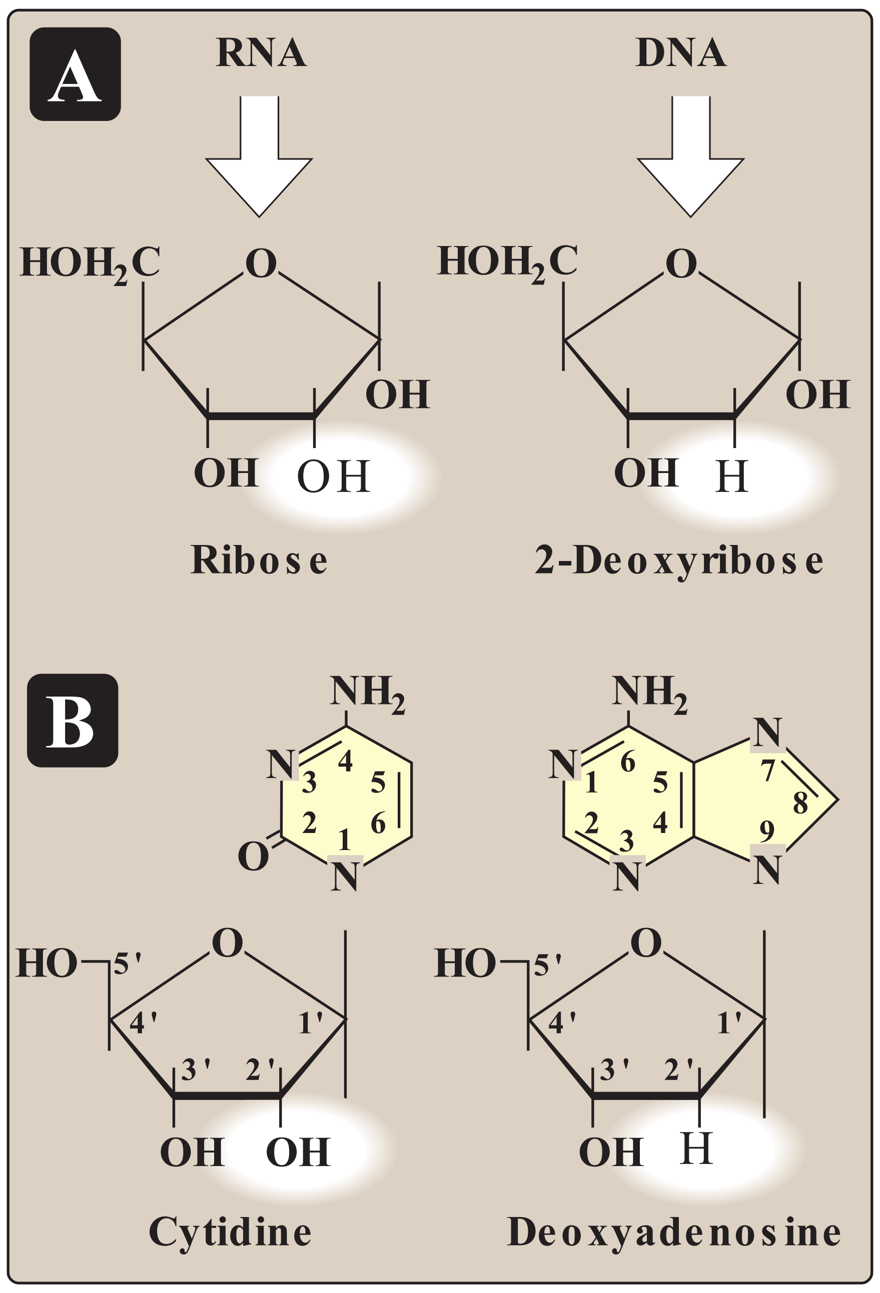


Figure 22.3
A. Pentoses found in nucleic acids.
B. Examples of the numbering systems for purine- and pyrimidine-containing nucleosides.

cytosine (C), but they differ in their second pyrimidine base: DNA contains thymine (T), whereas RNA contains uracil (U). T and U differ in that only T has a methyl group (Figure 22.1). Unusual (modified) bases are occasionally found in some species of DNA and RNA (for example, in some viral DNA) and in transfer RNA (tRNA). Base modifications include methylation, glycosylation, acetylation, and reduction. Some examples of unusual bases are shown in Figure 22.2. [Note: The presence of an unusual base in a nucleotide sequence may aid in its recognition by specific enzymes or protect it from being degraded by nucleases.]

B. Nucleosides

The addition of a pentose sugar to a base through a glycosidic bond produces a nucleoside. If the sugar is ribose, a ribonucleoside is produced, and if the sugar is 2-deoxyribose, a deoxyribonucleoside is produced (Figure 22.3A). The ribonucleosides of A, G, C, and U are named adenosine, guanosine, cytidine, and uridine, respectively. The deoxyribonucleosides of A, G, C, and T have the added prefix, "deoxy-" (for example, deoxyadenosine). [Note: The compound deoxythymidine is often simply called thymidine, with the "deoxy-" prefix being understood, because it is incorporated into DNA only.] The carbon and nitrogen atoms in the rings of the base and the sugar are numbered separately (Figure 22.3B). Note that the carbons in the pentose are numbered 1' to 5'. Thus, when the 5'-carbon of a nucleoside (or nucleotide) is referred to, a carbon atom in the pentose, rather than an atom in the base, is being specified.

C. Nucleotides

The addition of one or more phosphate groups to a nucleoside produces a nucleotide. The first phosphate group is attached by an ester linkage to the 5'-OH of the pentose, forming a nucleoside 5'-phosphate or a 5'-nucleotide. The type of pentose is denoted by the prefix in the names "5'-ribonucleotide" and "5'-deoxyribonucleotide." If one phosphate group is attached to the 5'-carbon of the pentose, the structure is a nucleoside monophosphate, like adenosine monophosphate [AMP] also called adenylate). If a second or third phosphate is added to the nucleoside, a nucleoside diphosphate (for example, adenosine diphosphate [ADP] or triphosphate (for example, adenosine triphosphate [ATP]) results (Figure 22.4). The second and third phosphates are each connected to the nucleotide by a "high-energy" bond. [Note: The phosphate groups are responsible for the negative charges associated with nucleotides and cause DNA and RNA to be referred to as "nucleic acids."]

III. SYNTHESIS OF PURINE NUCLEOTIDES

The atoms of the purine ring are contributed by a number of compounds, including amino acids (aspartate, glycine, and glutamine), CO_2, and N^{10}-formyltetrahydrofolate (Figure 22.5). The purine ring is constructed primarily in the liver by a series of reactions that add the donated carbons and nitrogens to a preformed ribose 5-phosphate. (See p. 147 for the synthesis of ribose 5-phosphate by the pentose phosphate pathway.)

A. Synthesis of 5-phosphoribosyl-1-pyrophosphate

5-Phosphoribosyl-1-pyrophosphate (PRPP) is an "activated pentose" that participates in the synthesis and salvage of purines and pyrimidines. Synthesis of PRPP from ATP and ribose 5-phosphate is catalyzed by PRPP synthetase (Figure 22.6). This X-linked enzyme is activated by inorganic phosphate and inhibited by purine nucleotides (end-product inhibition). [Note: The sugar moiety of PRPP is ribose, and, therefore, ribonucleotides are the end products of de novo purine synthesis. When deoxyribonucleotides are required for DNA synthesis, the ribose sugar moiety is reduced (see p. 297).]

B. Synthesis of 5-phosphoribosylamine

Synthesis of 5-phosphoribosylamine from PRPP and glutamine is shown in Figure 22.7. The amide group of glutamine replaces the pyrophosphate group attached to carbon 1 of PRPP. This is the committed step in purine nucleotide biosynthesis. The enzyme, glutamine:phosphoribosylpyrophosphate amidotransferase, is inhibited by the purine 5'-nucleotides AMP and guanosine monophosphate ([GMP] also called guanylate), the end products of the pathway. The rate of the reaction is also controlled by the intracellular concentration of PRPP. [Note: The concentration of PRPP is normally far below the Michaelis constant (K_m) for the amidotransferase. Therefore, any small change in the PRPP concentration causes a proportional change in rate of the reaction (see p. 59).]

C. Synthesis of inosine monophosphate, the "parent" purine nucleotide

The next nine steps in purine nucleotide biosynthesis leading to the synthesis of inosine monophosphate ([IMP] whose base is hypoxanthine) are illustrated in Figure 22.7. Four steps in this pathway require ATP as an energy source, and two steps in the pathway require N^{10}-formyltetrahydrofolate as a one-carbon donor (see p. 267). [Note: Hypoxanthine is found in tRNA (see p. 437).]

D. Synthetic inhibitors of purine synthesis

Some synthetic inhibitors of purine synthesis (for example, the sulfonamides), are designed to inhibit the growth of rapidly dividing microorganisms without interfering with human cell functions (see Figure 22.7). Other purine synthesis inhibitors, such as structural analogs of

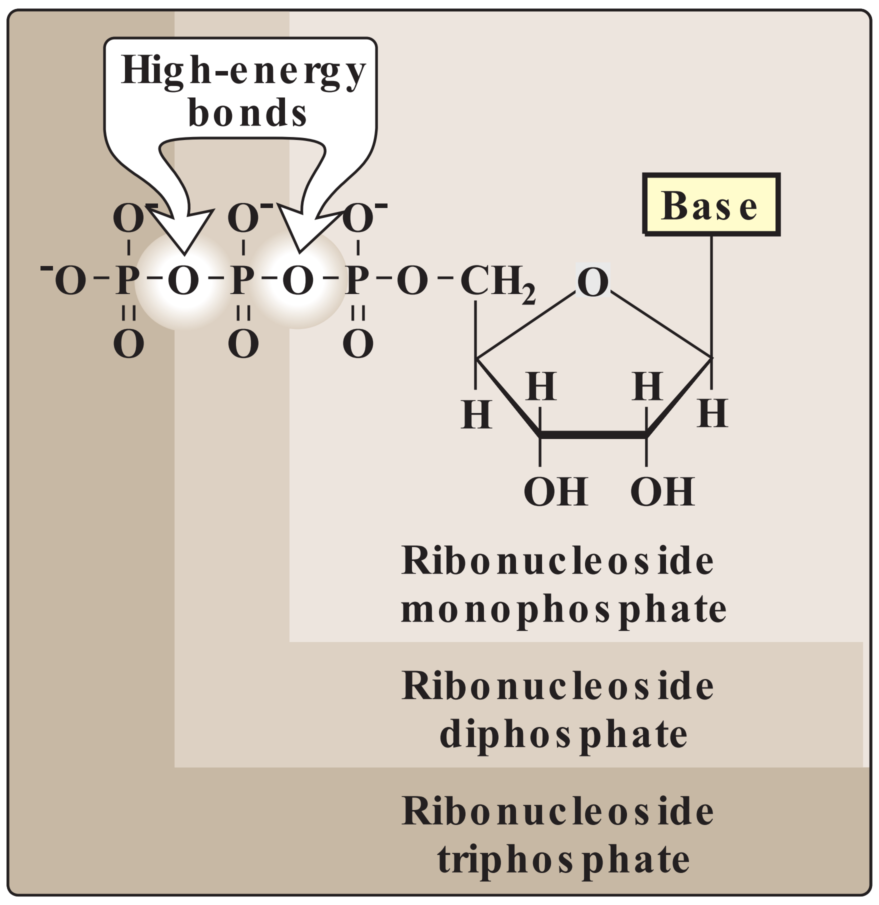

High-energy bonds
Base
Ribonucleoside monophosphate
Ribonucleoside diphosphate
Ribonucleoside triphosphate

Figure 22.4
Ribonucleoside monophosphate, diphosphate, and triphosphate.

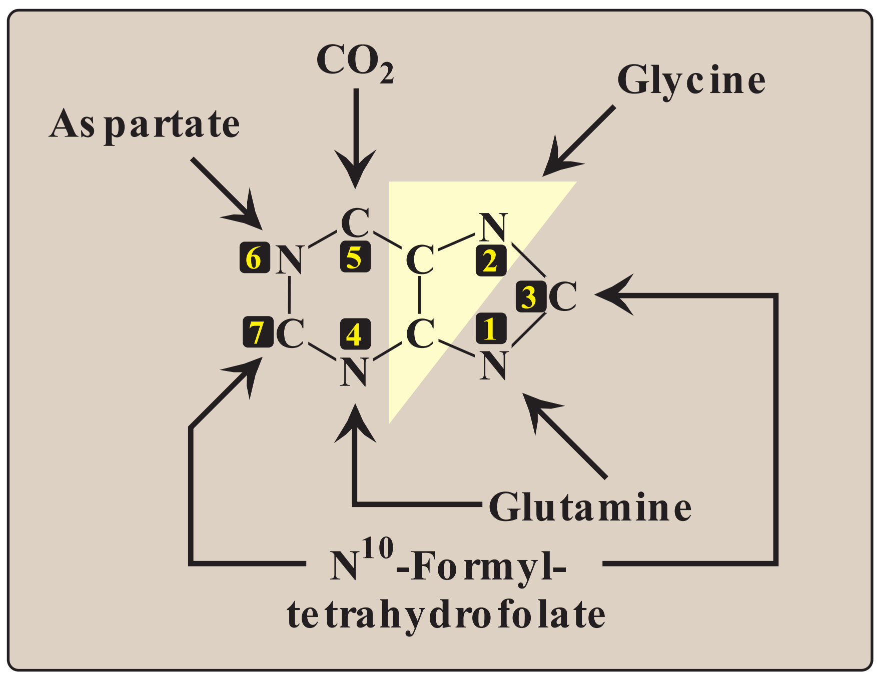

CO$_2$
Glycine
Aspartate
Glutamine
N^{10}-Formyl-tetrahydrofolate
1
2
3
4
5
6
7

Figure 22.5
Sources of the individual atoms in the purine ring. The order in which the atoms are added is shown by the numbers in the black boxes (see Figure 22.7).

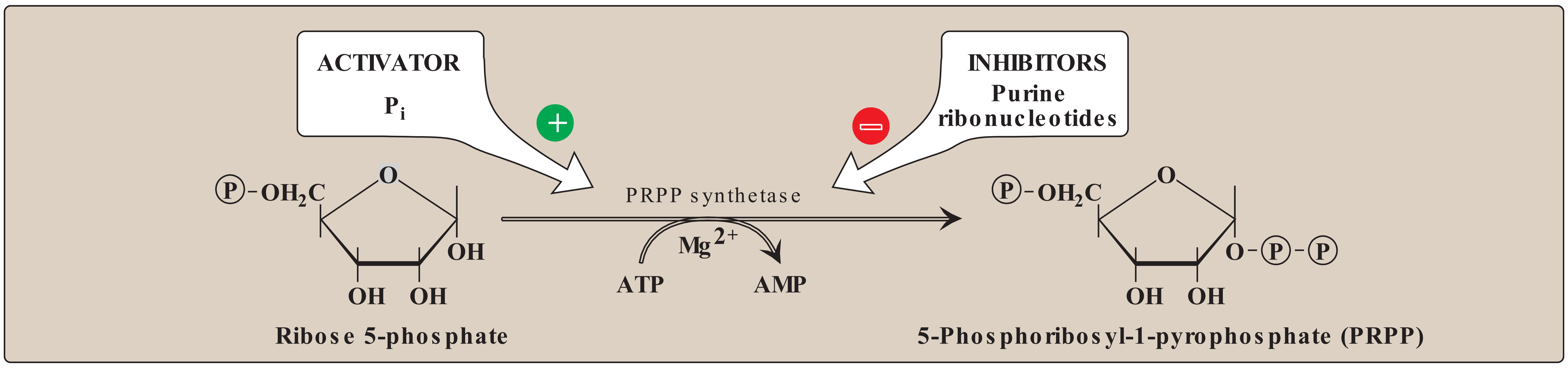
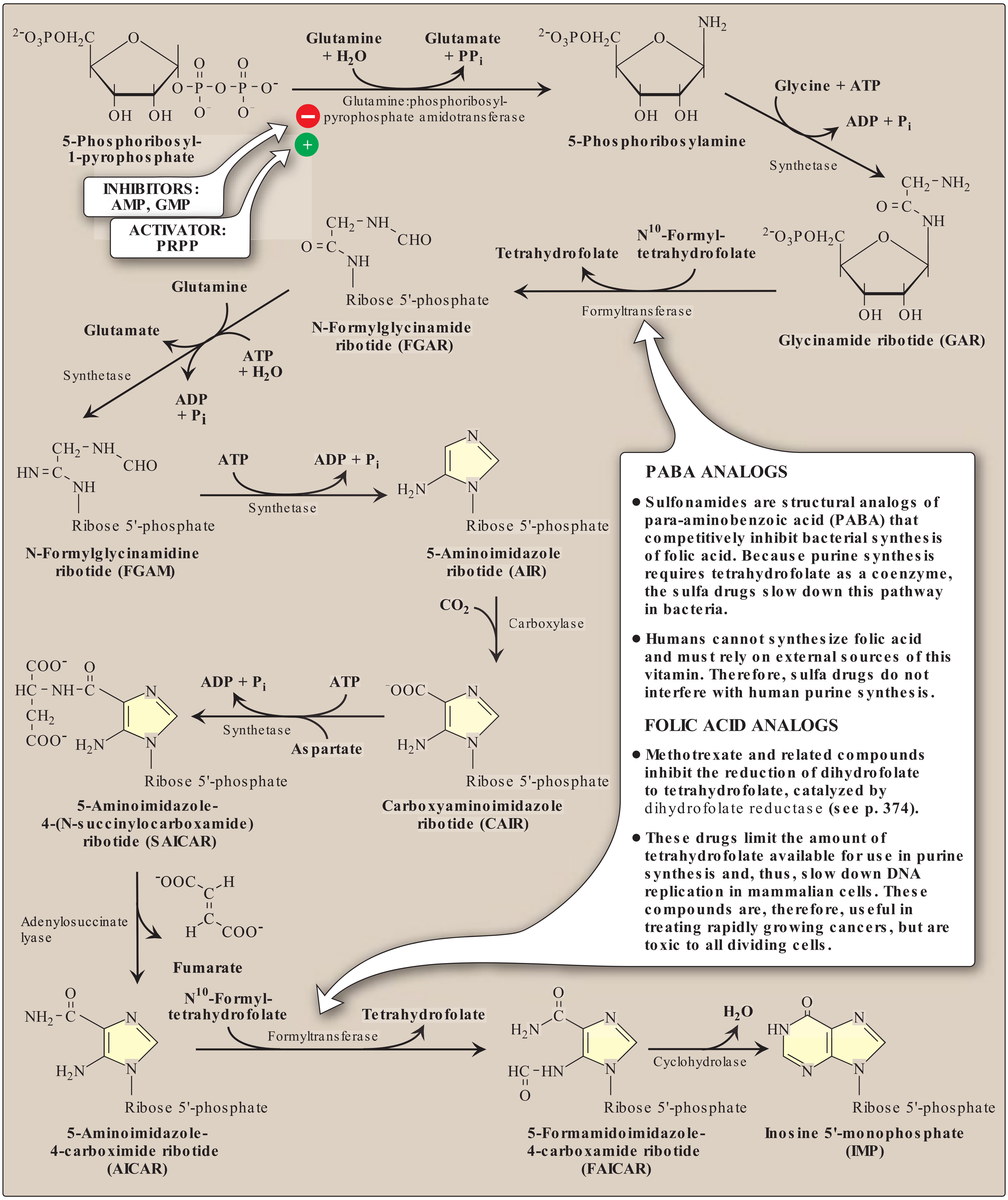

ACTIVATOR
P$_i$
INHIBITORS
Purine ribonucleotides
PRPP synthetase
Mg^{2+}
ATP
AMP
Ribose 5-phosphate
5-Phosphoribosyl-1-pyrophosphate (PRPP)

Figure 22.6
Synthesis of PRPP, showing the activator and inhibitors of the reaction. [Note: This is not the committed step of purine synthesis because PRPP is used in other pathways.] Ⓟ= phosphate; P_i = inorganic phosphate; AMP = adenosine monophosphate.


5-Phosphoribosyl-1-pyrophosphate
Glutamine + H_2O
Glutamate + PP_i
Glutamine:phosphoribosylpyrophosphate amidotransferase
INHIBITORS: AMP, GMP
ACTIVATOR: PRPP
5-Phosphoribosylamine
Glycine + ATP
ADP + P_i
Synthetase
Glycinamide ribotide (GAR)
N^{10}-Formyltetrahydrofolate
Tetrahydrofolate
Formyltransferase
N-Formylglycinamide ribotide (FGAR)
Ribose 5'-phosphate
Glutamine
Glutamate
ATP + H_2O
Synthetase
ADP + P_i
N-Formylglycinamidine ribotide (FGAM)
ATP
ADP + P_i
Synthetase
5-Aminoimidazole ribotide (AIR)
CO_2
Carboxylase
Carboxyaminoimidazole ribotide (CAIR)
ATP
ADP + P_i
Synthetase
Aspartate
5-Aminoimidazole-4-(N-succinylocarboxamide) ribotide (SAICAR)
Adenylosuccinate lyase
Fumarate
5-Aminoimidazole-4-carboximide ribotide (AICAR)
N^{10}-Formyltetrahydrofolate
Tetrahydrofolate
Formyltransferase
5-Formamidoimidazole-4-carboxamide ribotide (FAICAR)
H_2O
Cyclohydrolase
Inosine 5'-monophosphate (IMP)

PABA ANALOGS

- Sulfonamides are structural analogs of para-aminobenzoic acid (PABA) that competitively inhibit bacterial synthesis of folic acid. Because purine synthesis requires tetrahydrofolate as a coenzyme, the sulfa drugs slow down this pathway in bacteria.
- Humans cannot synthesize folic acid and must rely on external sources of this vitamin. Therefore, sulfa drugs do not interfere with human purine synthesis.

FOLIC ACID ANALOGS

- Methotrexate and related compounds inhibit the reduction of dihydrofolate to tetrahydrofolate, catalyzed by dihydrofolate reductase (see p. 374).
- These drugs limit the amount of tetrahydrofolate available for use in purine synthesis and, thus, slow down DNA replication in mammalian cells. These compounds are, therefore, useful in treating rapidly growing cancers, but are toxic to all dividing cells.

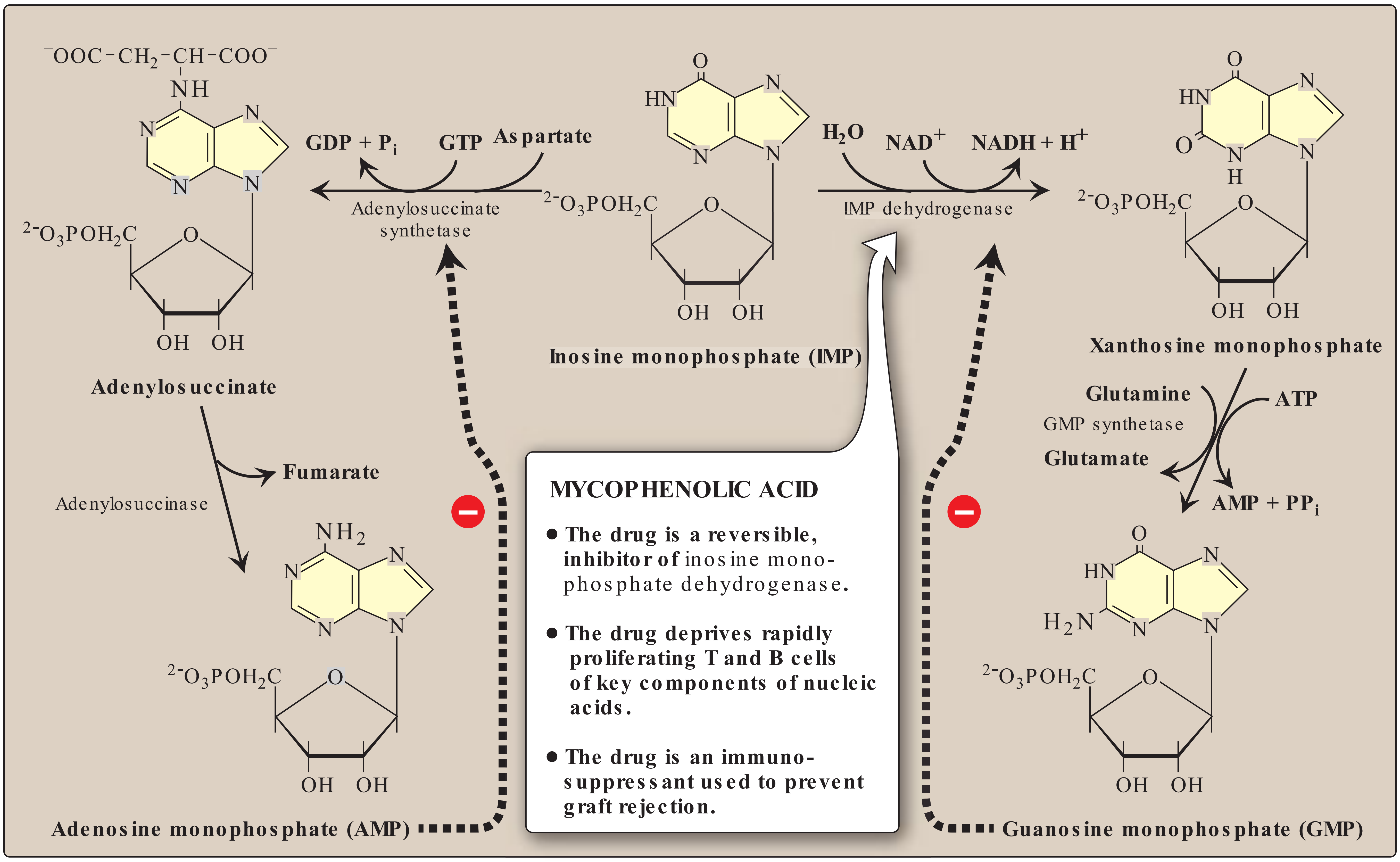
Figure 22.7
De novo synthesis of purine nucleotides, showing the inhibitory effect of some structural analogs. AMP = adenosine monophosphate; ADP = adenosine diphosphate; GMP = guanosine monophosphate; PRPP = 5-phosphoribosyl-1-pyrophosphate; P_i = inorganic phosphate; PP_i = pyrophosphate.

folic acid (for example, methotrexate), are used pharmacologically to control the spread of cancer by interfering with the synthesis of nucleotides and, therefore, of DNA and RNA (see Figure 22.7).

> Inhibitors of human purine synthesis are extremely toxic to tissues, especially to developing structures such as in a fetus, or to cell types that normally replicate rapidly, including those of bone marrow, skin, gastrointestinal (GI) tract, immune system, or hair follicles. As a result, individuals taking such anticancer drugs can experience adverse effects, including anemia, scaly skin, GI tract disturbance, immunodeficiencies, and hair loss.

E. Synthesis of adenosine and guanosine monophosphate

The conversion of IMP to either AMP or GMP uses a two-step, energy-requiring pathway (Figure 22.8). Note that the synthesis of AMP requires guanosine triphosphate (GTP) as an energy source, whereas the synthesis of GMP requires ATP. Also, the first reac-



$^{-}OOC-CH_2-CH-COO^{-}$
NH
$^{2-}O_3POH_2C$
OH OH
Adenylosuccinate
GDP + P_i
GTP
Aspartate
Adenylosuccinate synthetase
$^{2-}O_3POH_2C$
OH OH
Inosine monophosphate (IMP)
H_2O
NAD^+
NADH + H^+
IMP dehydrogenase
$^{2-}O_3POH_2C$
OH OH
Xanthosine monophosphate
Glutamine
ATP
GMP synthetase
Glutamate
AMP + PP_i
Fumarate
Adenylosuccinase
NH_2
$^{2-}O_3POH_2C$
OH OH
Adenosine monophosphate (AMP)
H_2N
$^{2-}O_3POH_2C$
OH OH
Guanosine monophosphate (GMP)

MYCOPHENOLIC ACID

- **The drug is a reversible, inhibitor of** inosine monophosphate dehydrogenase.
- **The drug deprives rapidly proliferating T and B cells of key components of nucleic acids.**
- **The drug is an immunosuppressant used to prevent graft rejection.**

Figure 22.8
Conversion of IMP to AMP and GMP showing feedback inhibition. [Note: AMP is also called adenylate. GMP is also called guanylate.] NAD(H) = nicotinamide adenine dinucleotide; GDP = guanosine diphosphate; GTP = guanosine triphosphate; AMP = adenosine monophosphate; P_i = inorganic phosphate; PP_i = pyrophosphate.

tion in each pathway is inhibited by the end product of that pathway. This provides a mechanism for diverting IMP to the synthesis of the purine present in lesser amounts. If both AMP and GMP are present in adequate amounts, the de novo pathway of purine synthesis is turned off at the amidotransferase step.

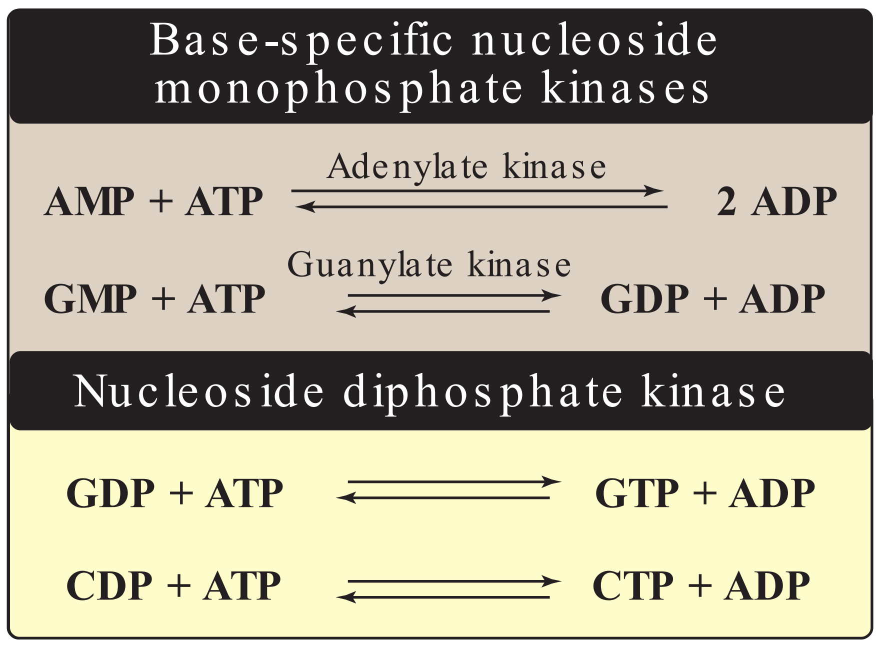


Figure 22.9
Conversion of nucleoside monophosphates to nucleoside diphosphates and triphosphates. AMP = adenosine monophosphate; ADP = adenosine diphosphate; GMP = guanosine monophosphate; GDP = guanosine diphosphate; GTP = guanosine triphosphate; CDP = cytidine diphosphate; CTP = cytidine triphosphate.

F. Conversion of nucleoside monophosphates to nucleoside diphosphates and triphosphates

Nucleoside diphosphates are synthesized from the corresponding nucleoside monophosphates by base-specific nucleoside monophosphate kinases (Figure 22.9). [Note: These kinases do not discriminate between ribose or deoxyribose in the substrate.] ATP is generally the source of the transferred phosphate because it is present in higher concentrations than the other nucleoside triphosphates. Adenylate kinase is particularly active in the liver and in muscle, where the turnover of energy from ATP is high. Its function is to maintain equilibrium among the adenine nucleotides (AMP, ADP, and ATP). Nucleoside diphosphates and triphosphates are interconverted by nucleoside diphosphate kinase, an enzyme that, unlike the monophosphate kinases, has broad substrate specificity.

G. Salvage pathway for purines

Purines that result from the normal turnover of cellular nucleic acids, or the small amount that is obtained from the diet and not degraded, can be converted to nucleoside triphosphates and used by the body. This is referred to as the “salvage pathway” for purines. [Note: Salvage is particularly important in the brain.]

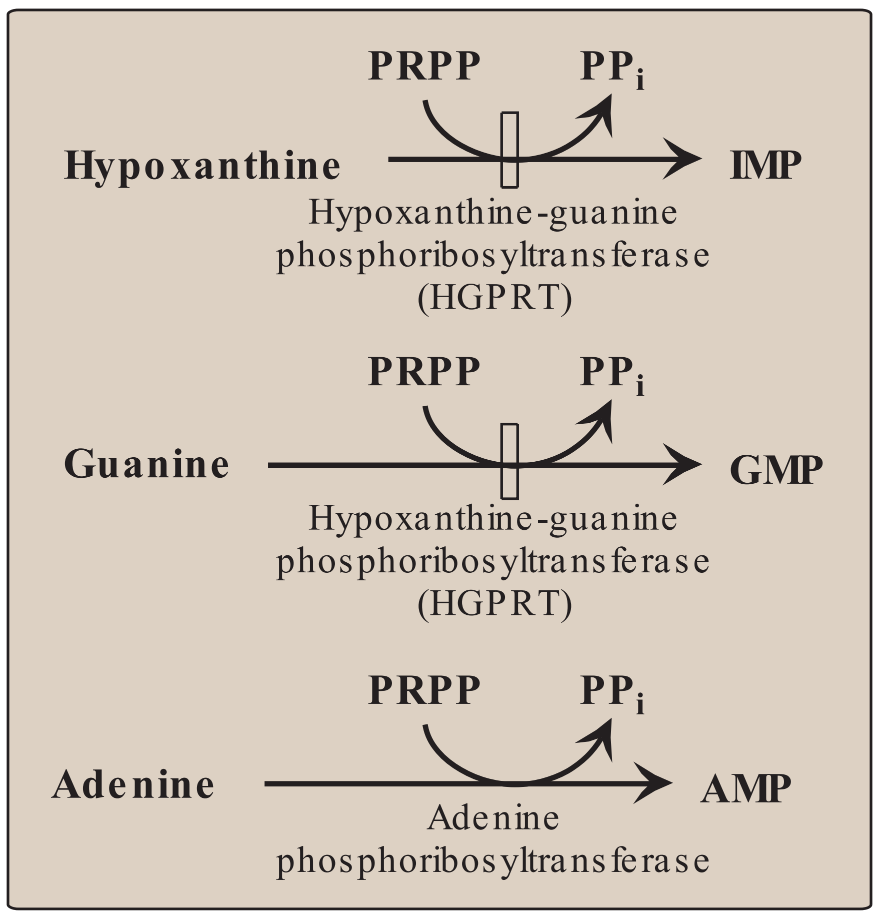

PRPP
PPi
Hypoxanthine
IMP
Hypoxanthine-guanine phosphoribosyltransferase (HGPRT)
PRPP
PPi
Guanine
GMP
Hypoxanthine-guanine phosphoribosyltransferase (HGPRT)
PRPP
PPi
Adenine
AMP
Adenine phosphoribosyltransferase

Figure 22.10
Salvage pathways of purine nucleotide synthesis. [Note: Virtually complete deficiency of HGPRT results in Lesch-Nyhan syndrome. Partial deficiencies of HGPRT are known. As the amount of functional enzyme increases, the severity of the symptoms decreases.] IMP = inosine monophosphate; GMP = guanosine monophosphate; AMP = adenosine monophosphate; PRPP = 5-phosphoribosyl-1-pyrophosphate; PP_i = pyrophosphate.

1. **Salvage of purine bases to nucleotides:** Two enzymes are involved: adenine phosphoribosyltransferase (APRT) and hypoxanthine-guanine phosphoribosyltransferase (HGPRT). Both enzymes use PRPP as the source of the ribose 5-phosphate group (Figure 22.10). The release of pyrophosphate and its subsequent hydrolysis by pyrophosphatase makes these reactions irreversible. [Note: Adenosine is the only purine nucleoside to be salvaged. It is phosphorylated to AMP by adenosine kinase.]

2. **Lesch-Nyhan syndrome:** Lesch-Nyhan is a rare, X-linked recessive disorder associated with a virtually complete deficiency of HGPRT. The deficiency results in an inability to salvage hypoxanthine or guanine, from which excessive amounts of uric acid, the end product of purine degradation, are then produced (see p. 298). In addition, the lack of this salvage pathway causes increased PRPP levels and decreased IMP and GMP levels. As a result, glutamine: phosphoribosylpyrophosphate amidotransferase (the regulated step in purine synthesis) has excess substrate and decreased inhibitors available, and de novo purine synthesis is increased. The combination of decreased purine reutilization and increased purine synthesis results in increased degradation of purines and the production of large amounts of uric acid, making Lesch-Nyhan a heritable cause of hyperuricemia. In patients with Lesch-Nyhan syndrome, the hyperuricemia frequently results in the formation of uric acid stones in the kidneys (urolithiasis) and the deposition of urate crystals in the joints (gouty arthritis) and soft tissues. In addition, the syndrome is characterized by motor dysfunction, cognitive deficits, and behav-

ioral disturbances that include self-mutilation (for example, biting of lips and fingers) as shown in Figure 22.11).

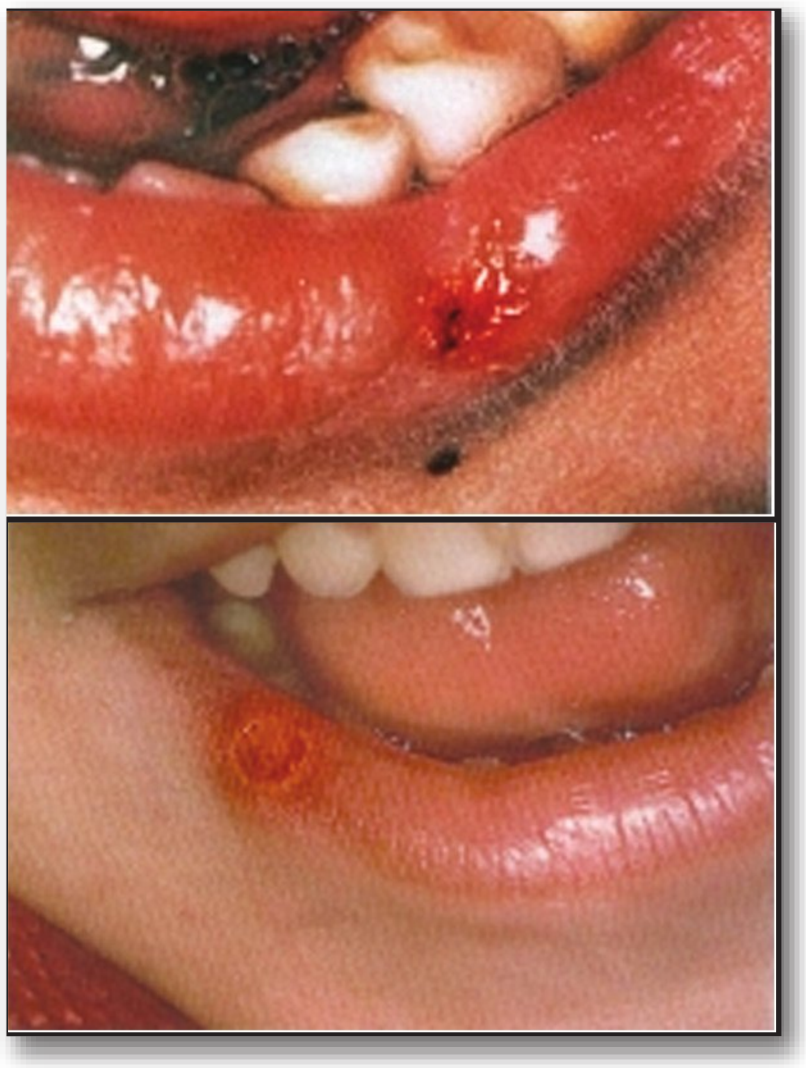


Figure 22.11
Lesions on the lips of Lesch-Nyhan patients caused by self-mutilation.

IV. SYNTHESIS OF DEOXYRIBONUCLEOTIDES

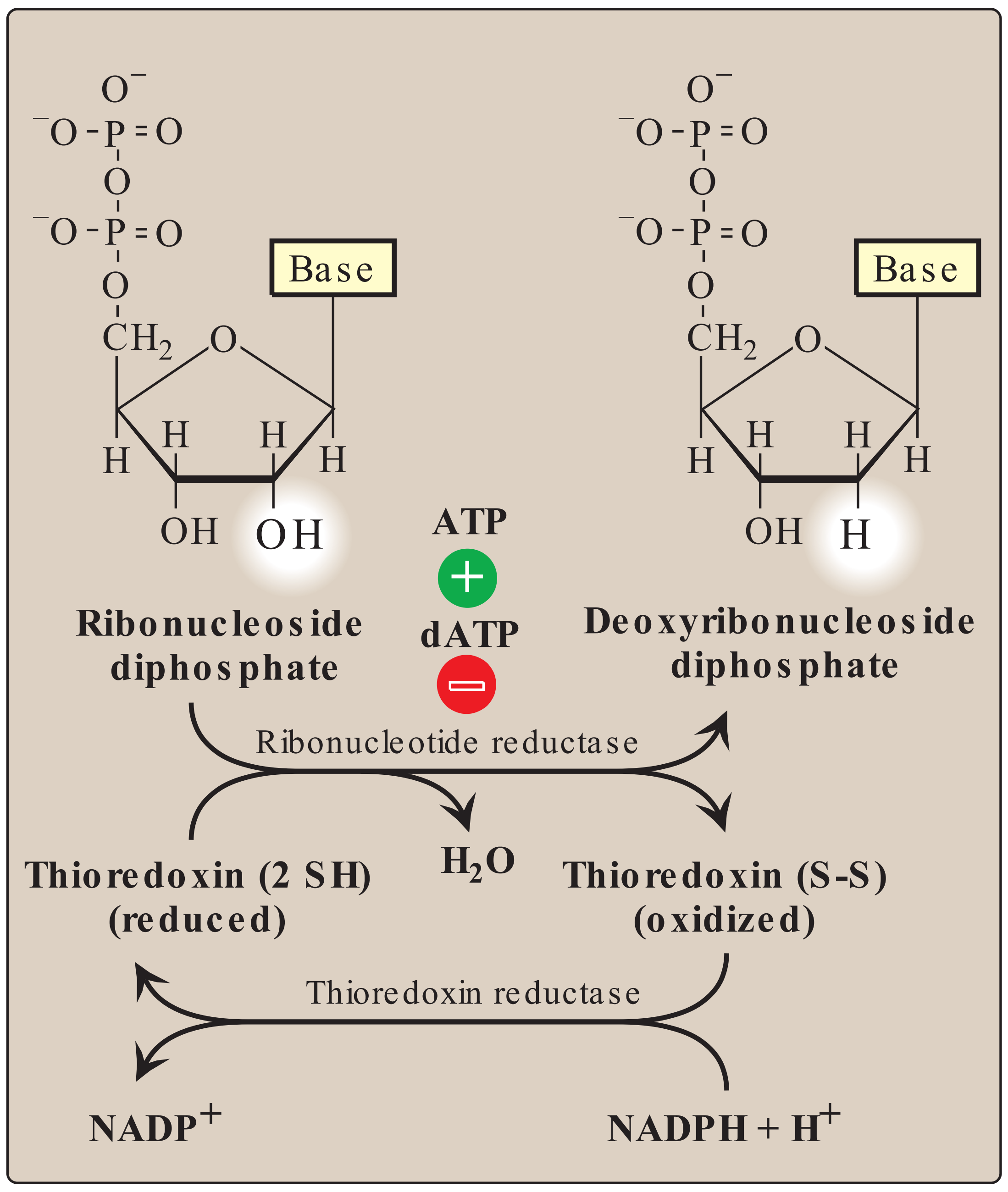
The nucleotides described thus far all contain ribose (ribonucleotides). The nucleotides required for DNA synthesis, however, are 2'-deoxyribonucleotides, which are produced from ribonucleoside diphosphates by the enzyme ribonucleotide reductase during the S-phase of the cell cycle (see p. 407). [Note: The same enzyme acts on pyrimidine ribonucleotides.]

A. Ribonucleotide reductase

Ribonucleotide reductase (ribonucleoside diphosphate reductase) is composed of two nonidentical dimeric subunits, R1 and R2, and is specific for the reduction of purine nucleoside diphosphates (ADP and GDP) and pyrimidine nucleoside diphosphates (CDP and UDP) to their deoxy forms (dADP, dGDP, dCDP, and dUDP). The immediate donors of the hydrogen atoms needed for the reduction of the 2'-hydroxyl group are two sulfhydryl groups on the enzyme itself, which, during the reaction, form a disulfide bond (Figure 22.12).

1. **Regeneration of reduced enzyme:** In order for ribonucleotide reductase to continue to produce deoxyribonucleotides, the disulfide bond created during the production of the 2'-deoxy carbon must be reduced. The source of the reducing equivalents for this purpose is thioredoxin, a peptide coenzyme of ribonucleotide reductase. Thioredoxin contains two cysteine residues separated by two amino acids in the peptide chain. The two sulfhydryl groups of thioredoxin donate their hydrogen atoms to ribonucleotide reductase, forming a disulfide bond in the process (see p. 19).

2. **Regeneration of reduced thioredoxin:** Thioredoxin must be converted back to its reduced form in order to continue to perform its function. The necessary reducing equivalents are provided by NADPH + H^+, and the reaction is catalyzed by thioredoxin reductase (see Figure 22.12).



Base
Base
OH OH
OH H
ATP
+
dATP
−
Ribonucleoside diphosphate
Deoxyribonucleoside diphosphate
Ribonucleotide reductase
Thioredoxin (2 SH) (reduced)
H_2O
Thioredoxin (S-S) (oxidized)
Thioredoxin reductase
$NADP^+$
NADPH + H^+

Figure 22.12
Conversion of ribonucleotides to deoxyribonucleotides. NADP(H) = nicotinamide adenine dinucleotide phosphate; dATP = deoxyadenosine triphosphate.

B. Regulation of deoxyribonucleotide synthesis

Ribonucleotide reductase is responsible for maintaining a balanced supply of the deoxyribonucleotides required for DNA synthesis. To achieve this, the regulation of the enzyme is complex. In addition to the catalytic (active) site, there are allosteric sites on the enzyme involved in regulating its activity (Figure 22.13).

1. **Activity sites:** The binding of dATP to allosteric sites (known as the activity sites) on the enzyme inhibits the overall catalytic activity of the enzyme and, therefore, prevents the reduction of any of the four nucleoside diphosphates. This effectively prevents DNA synthesis and explains the toxicity of increased levels of dATP seen in conditions such as

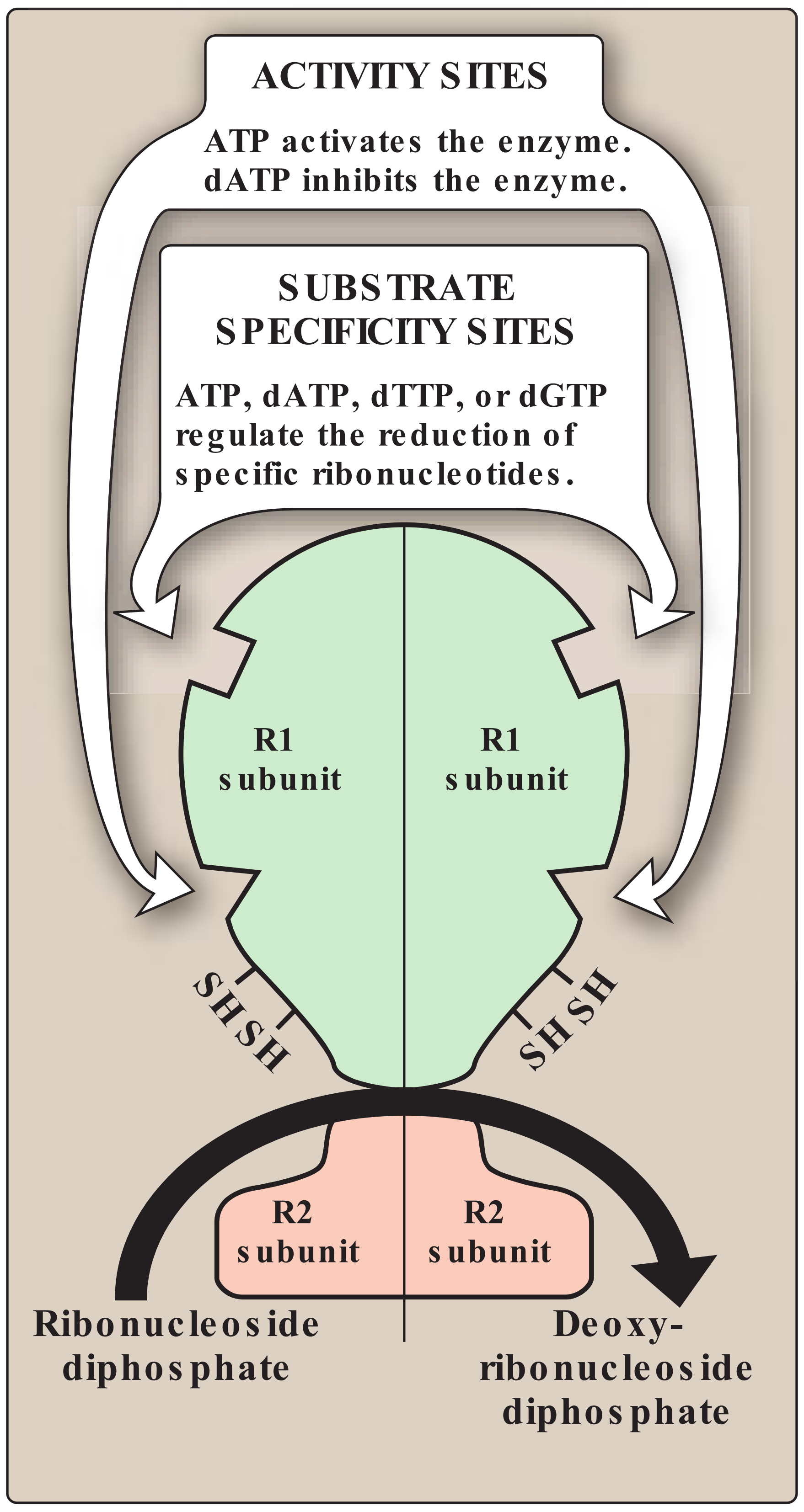

ACTIVITY SITES
ATP activates the enzyme.
dATP inhibits the enzyme.
SUBSTRATE SPECIFICITY SITES
ATP, dATP, dTTP, or dGTP regulate the reduction of specific ribonucleotides.
R1 subunit
R1 subunit
SH SH
SH SH
R2 subunit
R2 subunit
Ribonucleoside diphosphate
Deoxy-ribonucleoside diphosphate

Figure 22.13
Regulation of ribonucleotide reductase. dATP = deoxyadenosine triphosphate; dTTP = deoxythymidine triphosphate; dGTP = deoxyguanosine triphosphate.

adenosine deaminase deficiency (see p. 301). In contrast, ATP bound to these sites activates the enzyme.

2. **Substrate specificity sites:** The binding of nucleoside triphosphates to additional allosteric sites (known as the substrate specificity sites) on the enzyme regulates substrate specificity, causing an increase in the conversion of different species of ribonucleotides to deoxyribonucleotides as they are required for DNA synthesis. For example, deoxythymidine triphosphate binding at the specificity sites causes a conformational change that allows reduction of GDP to dGDP at the catalytic site.

The drug hydroxyurea (hydroxycarbamide) inhibits ribonucleotide reductase, thereby inhibiting the generation of substrates for DNA synthesis. Hydroxyurea is an antineoplastic agent and is used in the treatment of cancers such as melanoma. Hydroxyurea is also used in the treatment of sickle cell disease (see p. 36). However, the increase in fetal hemoglobin seen with hydroxyurea is not due to its effect on ribonucleotide reductase.

V. DEGRADATION OF PURINE NUCLEOTIDES

Degradation of dietary nucleic acids occurs in the small intestine, where a family of pancreatic enzymes hydrolyzes the nucleic acids to nucleotides. Inside the intestinal mucosal cells, purine nucleotides are sequentially degraded by specific enzymes to nucleosides and free bases, with uric acid as the end product of this pathway. [Note: Purine nucleotides from de novo synthesis are degraded in the liver primarily. The free bases are sent out from liver and salvaged by peripheral tissues.]

A. Degradation of dietary nucleic acids in the small intestine

Ribonucleases and deoxyribonucleases, secreted by the pancreas, hydrolyze dietary RNA and DNA to oligonucleotides. Oligonucleotides are further hydrolyzed by pancreatic phosphodiesterases, producing a mixture of 3'- and 5'-mononucleotides. In the intestinal mucosal cells, a family of nucleotidases removes the phosphate groups hydrolytically, releasing nucleosides that are further degraded by nucleosidases (nucleoside phosphorylases) to free bases plus (deoxy) ribose 1-phosphate. Dietary purine bases are not used to any appreciable extent for the synthesis of tissue nucleic acids. Instead, they are generally converted to uric acid in intestinal mucosal cells. Most of the uric acid enters the blood and is eventually excreted in the urine. A summary of this pathway is shown in Figure 22.14. [Note: Mammals other than primates express urate oxidase (uricase) which cleaves the purine ring, generating allantoin. Modified recombinant urate oxidase is now used clinically to lower urate levels.]

B Formation of uric acid

A summary of the steps in the production of uric acid and genetic diseases associated with deficiencies of specific degradative enzymes are shown in Figure 22.15. [Note: The bracketed numbers refer to specific reactions in the figure.]

[1] An amino group is removed from AMP to produce IMP by AMP deaminase or from adenosine to produce inosine (hypoxanthine-ribose) by adenosine deaminase.

[2] IMP and GMP are converted into their nucleoside forms (inosine and guanosine) by the action of 5'-nucleotidase.

[3] Purine nucleoside phosphorylase converts inosine and guanosine into their respective purine bases, hypoxanthine and guanine. [Note: A mutase interconverts ribose 1- and ribose 5-phosphate.]

[4] Guanine is deaminated to form xanthine.

[5] Hypoxanthine is oxidized by xanthine oxidase to xanthine, which is further oxidized by xanthine oxidase to uric acid, the final product of human purine degradation. Uric acid is excreted primarily in the urine.

C. Diseases associated with purine degradation

1. **Gout:** Gout is a disorder initiated by high levels of uric acid (the end product of purine catabolism) in blood (hyperuricemia), as a result of either the overproduction or underexcretion of uric acid. The hyperuricemia can lead to the deposition of monosodium urate (MSU) crystals in the joints and an inflammatory response to the crystals, causing first acute and then progressing to chronic gouty arthritis. Nodular masses of MSU crystals (tophi) may be deposited in the soft tissues, resulting in chronic tophaceous gout (Figure 22.16). Formation of uric acid stones in the kidney (urolithiasis) may also be seen. [Note: Hyperuricemia, while necessary, is not sufficient to cause gout, but gout is always preceded by hyperuricemia. Hyperuricemia is typically asymptomatic but may be indicative of comorbid conditions such as hypertension.] The definitive diagnosis of gout requires aspiration and examination of synovial fluid (Figure 22.17) from an affected joint (or material from a tophus) using polarized light microscopy to confirm the presence of needle-shaped MSU crystals (Figure 22.18).

   a. **Underexcretion of uric acid:** In over 90% of individuals, hyperuricemia is caused by underexcretion of uric acid. Underexcretion can be primary, due to as-yet-unidentified inherent excretory defects, or secondary to known disease processes that affect how the kidney handles urate (for example, in lactic acidosis, lactate increases renal urate reabsorption, thereby decreasing its excretion) and to environmental factors such as the use of drugs (for example, thiazide diuretics) or exposure to lead (saturnine gout).

   b. **Overproduction of uric acid:** A less common cause of hyperuricemia is from the overproduction of uric acid. Primary hyperuricemia is, for the most part, idiopathic (having no known cause). However, several identified mutations in the gene for X-linked PRPP synthetase result in the enzyme having an increased maximal velocity (V_{max}) (see p. 58) for the production of PRPP, a lower K_m (see p. 59) for ribose 5-phosphate, or a decreased sensitivity to purine nucleotides, its allosteric inhibitors (see p. 62). In each case, increased availability of PRPP increases

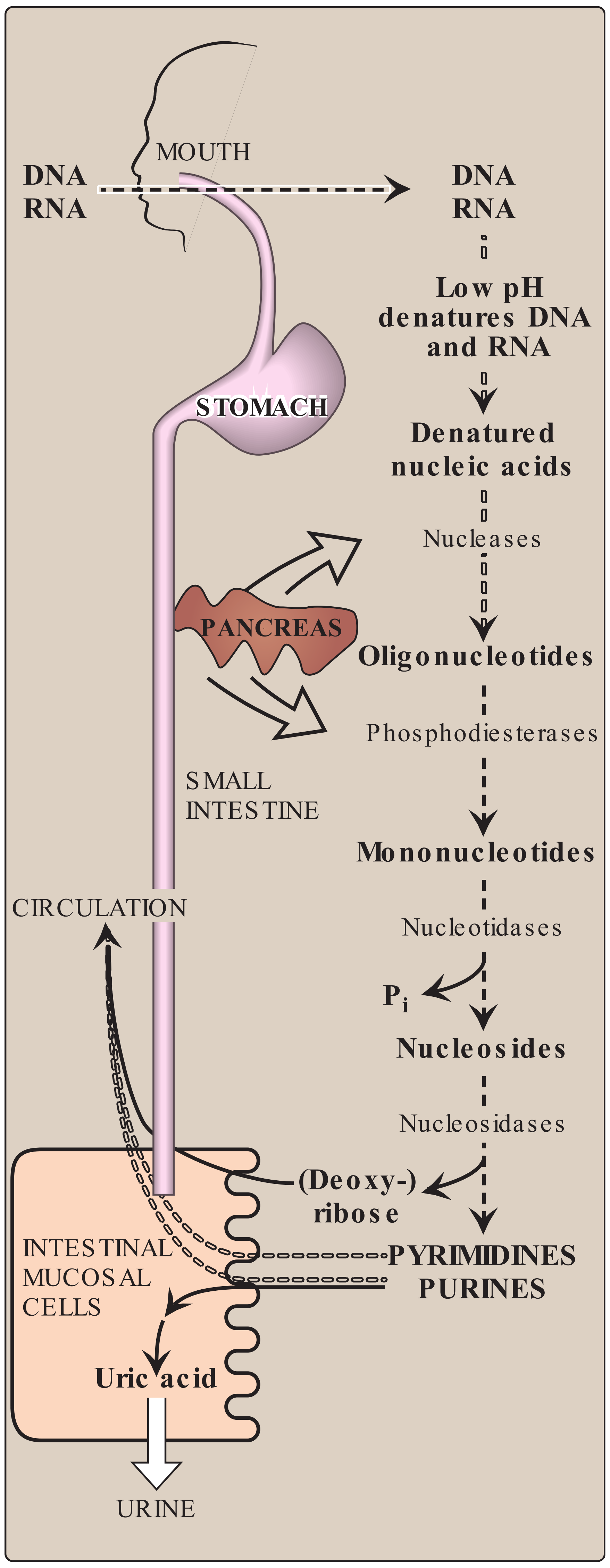
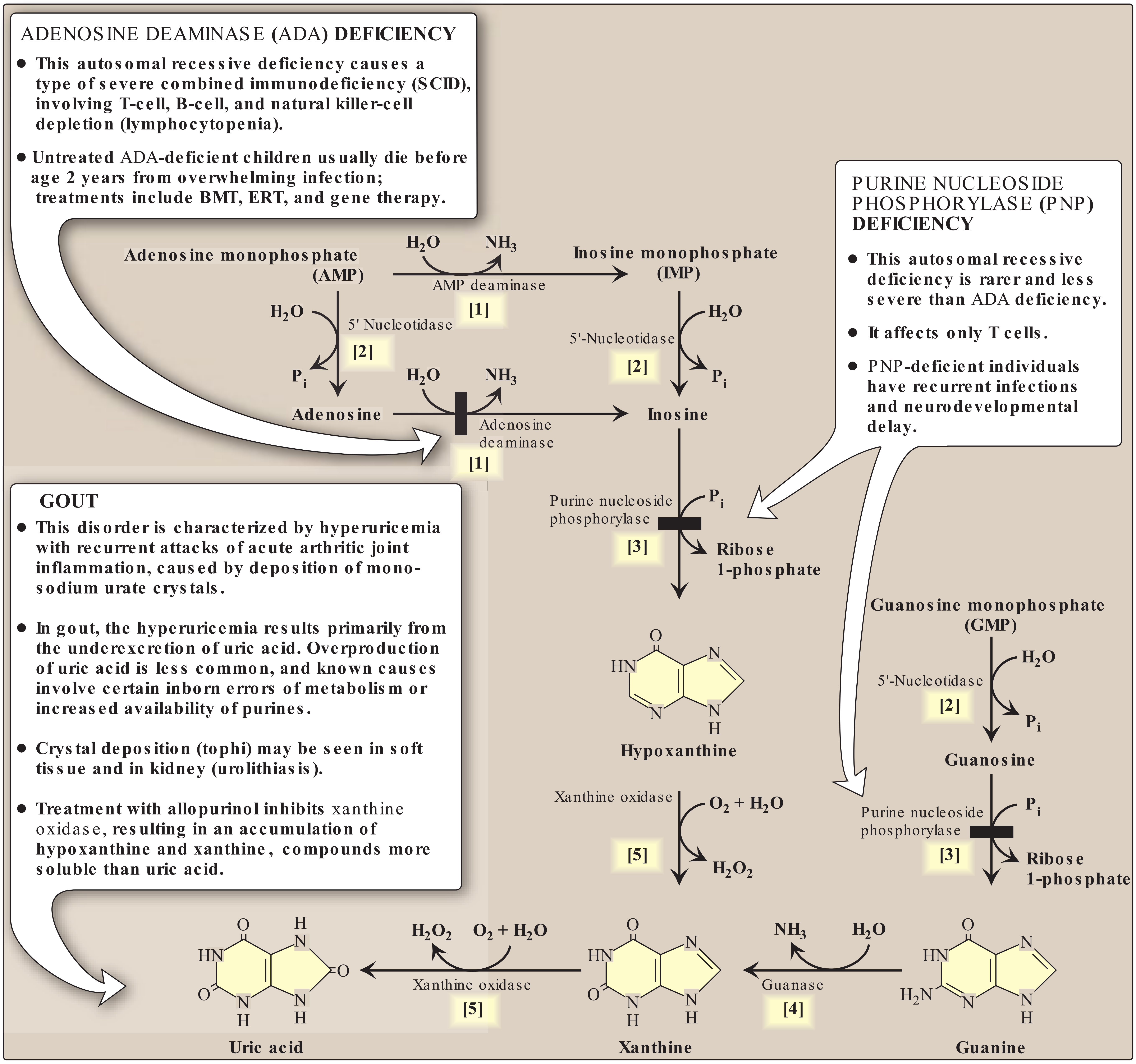


Figure 22.14
Digestion of dietary nucleic acids. [Note: Much of the metabolism of the mononucleotides occurs within the intestinal mucosal cells.]
P_i = inorganic phosphate.



ADENOSINE DEAMINASE (ADA) **DEFICIENCY**

- **This autosomal recessive deficiency causes a type of severe combined immunodeficiency (SCID), involving T-cell, B-cell, and natural killer-cell depletion (lymphocytopenia).**
- **Untreated** ADA**-deficient children usually die before age 2 years from overwhelming infection; treatments include BMT, ERT, and gene therapy.**

PURINE NUCLEOSIDE PHOSPHORYLASE (PNP) **DEFICIENCY**

- **This autosomal recessive deficiency is rarer and less severe than** ADA **deficiency.**
- **It affects only T cells.**
- PNP**-deficient individuals have recurrent infections and neurodevelopmental delay.**

GOUT

- **This disorder is characterized by hyperuricemia with recurrent attacks of acute arthritic joint inflammation, caused by deposition of monosodium urate crystals.**
- **In gout, the hyperuricemia results primarily from the underexcretion of uric acid. Overproduction of uric acid is less common, and known causes involve certain inborn errors of metabolism or increased availability of purines.**
- **Crystal deposition (tophi) may be seen in soft tissue and in kidney (urolithiasis).**
- **Treatment with allopurinol inhibits** xanthine oxidase, **resulting in an accumulation of hypoxanthine and xanthine, compounds more soluble than uric acid.**

Adenosine monophosphate (AMP)
H2O
NH3
AMP deaminase
[1]
Inosine monophosphate (IMP)
H2O
5' Nucleotidase
[2]
Pi
5'-Nucleotidase
[2]
H2O
Pi
H2O
NH3
Adenosine
Adenosine deaminase
[1]
Inosine
Purine nucleoside phosphorylase
[3]
Pi
Ribose 1-phosphate
Hypoxanthine
Guanosine monophosphate (GMP)
5'-Nucleotidase
[2]
H2O
Pi
Guanosine
Purine nucleoside phosphorylase
[3]
Pi
Ribose 1-phosphate
Xanthine oxidase
[5]
O2 + H2O
H2O2
H2O2
O2 + H2O
Xanthine oxidase
[5]
NH3
H2O
Guanase
[4]
Uric acid
Xanthine
Guanine
O
HN
N
N
H
H2N

Figure 22.15
The degradation of purine nucleotides to uric acid, illustrating some of the genetic diseases associated with this pathway. [Note: The numbers in brackets refer to the corresponding numbered citations in the text.] BMT = bone marrow transplantation; ERT = enzyme replacement therapy; P_i = inorganic phosphate.

purine production, resulting in elevated levels of plasma uric acid. Lesch-Nyhan syndrome (see p. 296) also causes hyperuricemia as a result of the decreased salvage of hypoxanthine and guanine and the subsequent increased availability of PRPP. Secondary hyperuricemia is typically the consequence of increased availability of purines (for example, in patients with myeloproliferative disorders or who are undergoing chemotherapy and so have a high rate of cell turnover). Hyperuricemia can also be the result of seemingly unrelated metabolic diseases, such as von Gierke disease (see Figure 11.8 on p. 130) or hereditary fructose intolerance (see p. 138).

> A diet rich in meat, seafood (particularly shellfish), and ethanol is associated with increased risk of gout, whereas a diet rich in low-fat dairy products is associated with a decreased risk.

c. **Treatment of gout:** Acute attacks of gout are treated with anti-inflammatory agents. Colchicine; steroidal drugs, such as prednisone; and nonsteroidal drugs, such as indomethacin, are used. [Note: Colchicine prevents formation of microtubules, thereby decreasing the movement of neutrophils into the affected area. Like the other anti-inflammatory drugs, it has no effect on uric acid levels.] Long-term therapeutic strategies for gout involve lowering the uric acid level below its saturation point (6.5 mg/dl), thereby preventing the deposition of urate crystals. Uricosuric agents, such as probenecid or sulfinpyrazone, that increase renal excretion of uric acid, are used in patients who are "underexcretors" of uric acid. Allopurinol, a structural analog of hypoxanthine, inhibits uric acid synthesis and is used in patients who are "overproducers" of uric acid. Allopurinol is converted in the body to oxypurinol, which inhibits xanthine oxidase (XO), resulting in an accumulation of hypoxanthine and xanthine (see Figure 22.15), compounds more soluble than uric acid and, therefore, less likely to initiate an inflammatory response. In patients with normal levels of HGPRT, the hypoxanthine can be salvaged, reducing the levels of PRPP and, therefore, de novo purine synthesis. Febuxostat, a nonpurine inhibitor of XO, is also available. [Note: Uric acid levels in the blood normally are close to the saturation point. One reason for this may be the strong antioxidant effects of uric acid.]

2. **Adenosine deaminase deficiency:** Adenosine deaminase (ADA) is expressed in a variety of tissues, but, in humans, lymphocytes have the highest activity of this cytoplasmic enzyme. A deficiency of ADA results in an accumulation of adenosine, which is converted to its ribonucleotide or deoxyribonucleotide forms by cellular kinases. As dATP levels rise, ribonucleotide reductase is inhibited, thereby preventing the production of all deoxyribose-containing nucleotides (see p. 297). Consequently, cells cannot make DNA and divide. [Note: The dATP and adenosine that accumulate in ADA deficiency lead to developmental arrest and apoptosis of lymphocytes.] In its most severe form, this autosomal-recessive disorder causes a type of severe combined immunodeficiency disease (SCID), involving a decrease in T cells, B cells, and natural killer cells. It is estimated that, in the United States, ADA deficiency accounts for approximately 14% of all cases of SCID. Treatments include bone marrow transplantation, enzyme replacement therapy, and gene therapy. Without appropriate treatment, children with this disorder usually die from infection by age 2 years. [Note: Purine nucleoside phosphorylase deficiency results in a less severe immunodeficiency primarily involving T cells.]

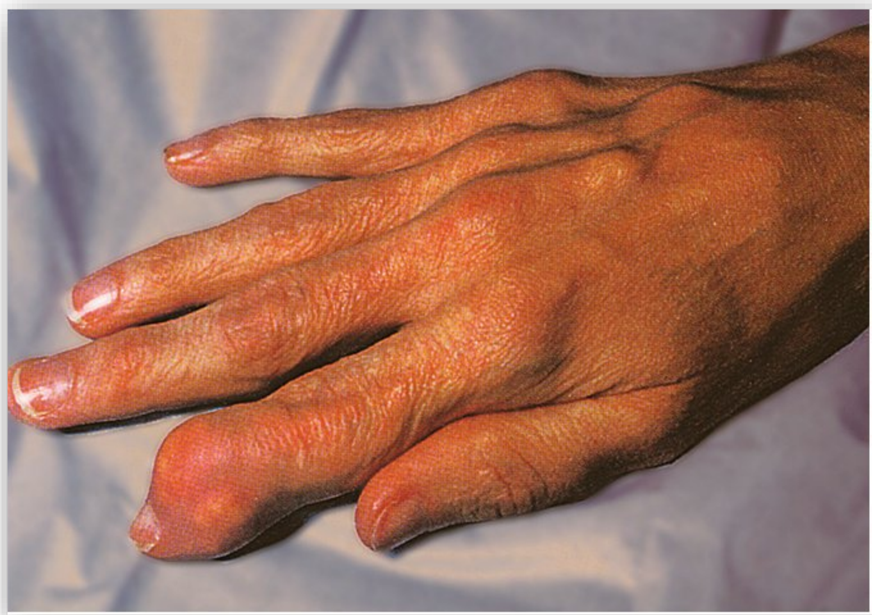
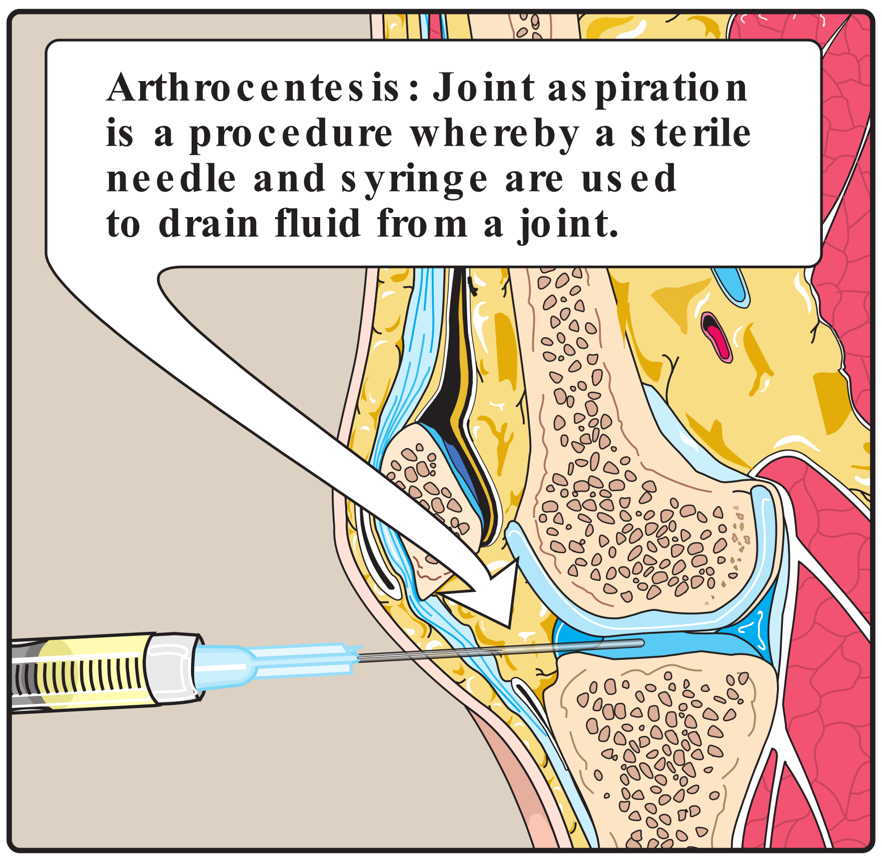


Figure 22.16
Tophaceous gout.



Arthrocentesis: Joint aspiration is a procedure whereby a sterile needle and syringe are used to drain fluid from a joint.

Figure 22.17
Analysis of joint fluid can help to define causes of joint swelling or arthritis, such as infection, gout, and rheumatoid disease.

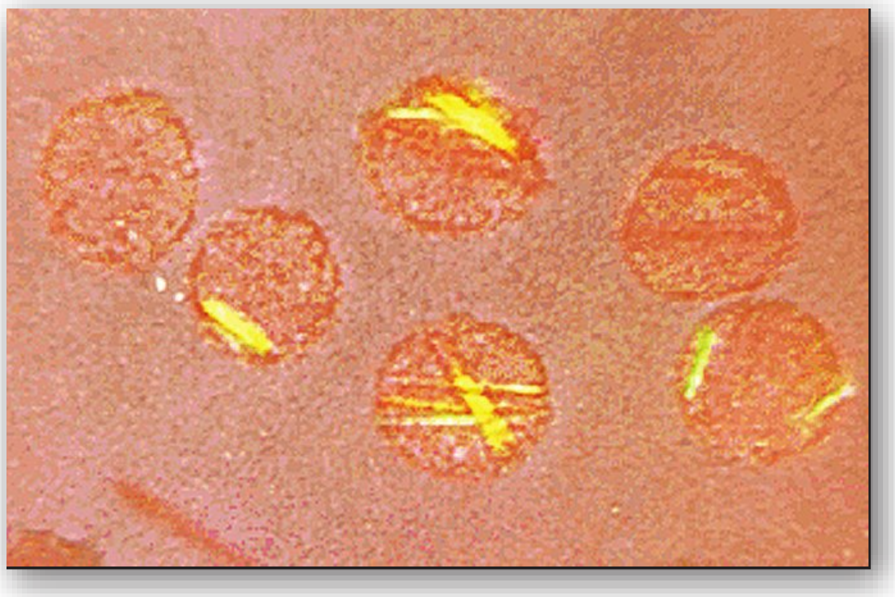


Figure 22.18
Gout can be diagnosed by the presence of negatively birefringent monosodium urate crystals in aspirated synovial fluid examined by polarized-light microscopy. Here, crystals are within polymorphonuclear leukocytes.

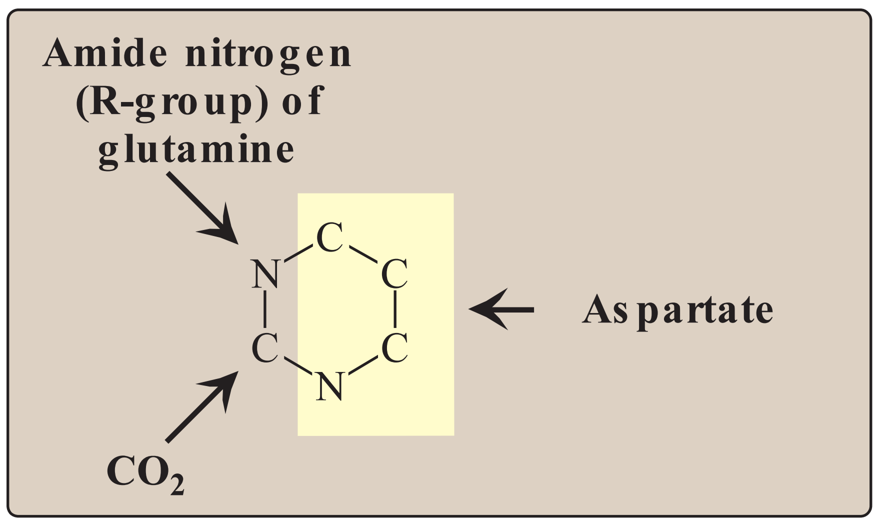


Figure 22.19
Sources of the individual atoms in the pyrimidine ring.

	CPS I	CPS II
Cellular location	**Mitochondria**	**Cytosol**
Pathway involved	**Urea cycle**	**Pyrimidine synthesis**
Source of nitrogen	**Ammonia**	**γ-Amide group of glutamine**
Regulators	**Activator: N-acetyl-glutamate**	**Activator: PRPP** **Inhibitor: UTP**

Figure 22.20
Summary of the differences between carbamoyl phosphate synthetase (CPS) I and II. PRPP = 5-phosphoribosyl-1-pyrophosphate; UTP = uridine triphosphate.

VI. PYRIMIDINE SYNTHESIS AND DEGRADATION

Unlike the synthesis of the purine ring, which is constructed on a pre-existing ribose 5-phosphate, the pyrimidine ring is synthesized before being attached to ribose 5-phosphate, which is donated by PRPP. The sources of the atoms in the pyrimidine ring are glutamine, CO_2, and aspartate (Figure 22.19).

A. Synthesis of carbamoyl phosphate

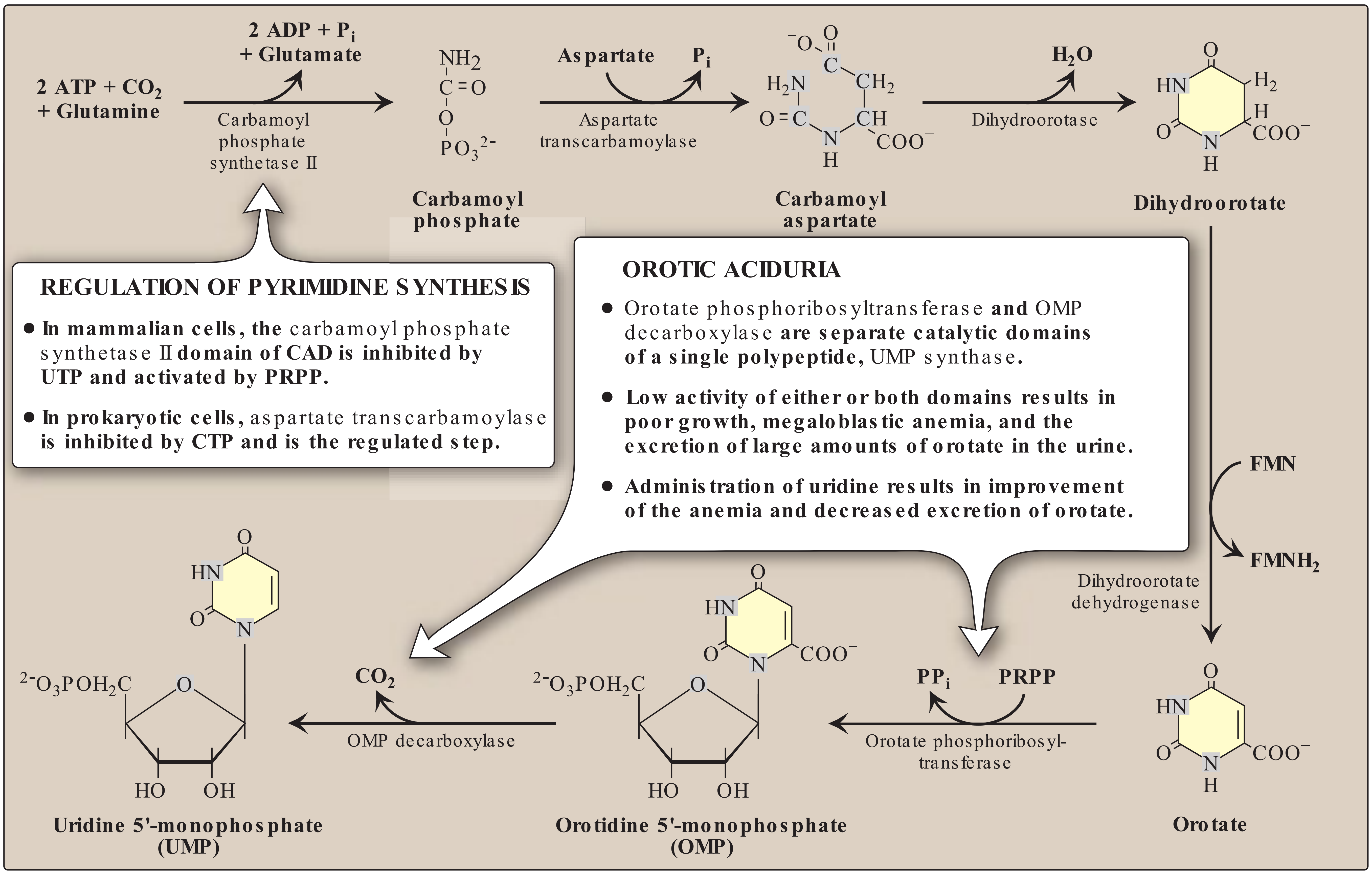
The regulated step of this pathway in mammalian cells is the synthesis of carbamoyl phosphate from glutamine and CO_2, catalyzed by carbamoyl phosphate synthetase (CPS) II. CPS II is inhibited by uridine triphosphate (the end product of this pathway, which can be converted into the other pyrimidine nucleotides), and is activated by PRPP. [Note: Carbamoyl phosphate, synthesized by CPS I, is also a precursor of urea (see p. 253). Defects in ornithine transcarbamylase of the urea cycle promote pyrimidine synthesis due to increased availability of carbamoyl phosphate. A comparison of the two enzymes is presented in Figure 22.20.]

B. Synthesis of orotic acid

The second step in pyrimidine synthesis is the formation of carbamoylaspartate, catalyzed by aspartate transcarbamoylase. The pyrimidine ring is then closed by dihydroorotase. The resulting dihydroorotate is oxidized to produce orotic acid (orotate) as shown in Figure 22.21. The enzyme that produces orotate, dihydroorotate dehydrogenase, is a flavoprotein associated with the inner mitochondrial membrane. All other enzymes in pyrimidine biosynthesis are cytosolic. [Note: The first three enzymic activities in this pathway (CPS II, aspartate transcarbamoylase, and dihydroorotase) are actually three different catalytic domains of a single polypeptide known as CAD from the first letter in the name of each domain. (See p. 19 for a discussion of domains.) This is an example of a multfunctional or multicatalytic polypeptide that facilitates the ordered synthesis of an important compound. Synthesis of the purine nucleotide IMP also involves multifunctional proteins.]

C. Formation of a pyrimidine nucleotide

The completed pyrimidine ring is converted to the nucleotide orotidine monophosphate (OMP) in the second stage of pyrimidine nucleotide synthesis (see Figure 22.21). PRPP is again the ribose 5-phosphate donor. The enzyme orotate phosphoribosyltransferase produces OMP and releases pyrophosphate, thereby making the reaction biologically irreversible. [Note: Both purine and pyrimidine synthesis require glutamine, aspartic acid, and PRPP as essential precursors.] OMP, the parent pyrimidine mononucleotide, is converted to uridine monophosphate (UMP) by orotidylate decarboxylase, which removes the carboxyl group. Orotate phosphoribosyltransferase and orotidylate decarboxylase are also catalytic domains of a single polypeptide chain called UMP synthase. Orotic aciduria (a very rare disorder) may be caused by a deficiency of one or both activities of this bifunctional enzyme, resulting in orotic acid in the urine (see Figure 22.21). UMP is sequentially phosphorylated to UDP and UTP. [Note: The UDP is a substrate for ribonucleotide reductase, which generates dUDP. The dUDP is phosphorylated to


2 ATP + CO2 + Glutamine
2 ADP + Pi + Glutamate
Carbamoyl phosphate synthetase II
Carbamoyl phosphate
Aspartate
Pi
Aspartate transcarbamoylase
Carbamoyl aspartate
H2O
Dihydroorotase
Dihydroorotate
FMN
FMNH2
Dihydroorotate dehydrogenase
Orotate
PPi
PRPP
Orotate phosphoribosyltransferase
Orotidine 5'-monophosphate (OMP)
CO2
OMP decarboxylase
Uridine 5'-monophosphate (UMP)

REGULATION OF PYRIMIDINE SYNTHESIS

- **In mammalian cells, the** carbamoyl phosphate synthetase II **domain of CAD is inhibited by UTP and activated by PRPP.**
- **In prokaryotic cells,** aspartate transcarbamoylase **is inhibited by CTP and is the regulated step.**

OROTIC ACIDURIA

- Orotate phosphoribosyltransferase **and** OMP decarboxylase **are separate catalytic domains of a single polypeptide,** UMP synthase**.**
- **Low activity of either or both domains results in poor growth, megaloblastic anemia, and the excretion of large amounts of orotate in the urine.**
- **Administration of uridine results in improvement of the anemia and decreased excretion of orotate.**

Figure 22.21
De novo pyrimidine synthesis. ADP = adenosine diphosphate; P_i = inorganic phosphate; $FMN(H_2)$ = flavin mononucleotide; CTP = cytidine triphosphate; PRPP = 5-phosphoribosyl-1-pyrophosphate; PP_i = pyrophosphate.

dUTP, which is rapidly hydrolyzed to dUMP by UTP diphosphatase (dUTPase). dUTPase, then, plays an important role in reducing availability of dUTP as a substrate for DNA synthesis, thereby preventing erroneous incorporation of uracil into DNA.]

D. Synthesis of cytidine triphosphate

Cytidine triphosphate (CTP) is produced by amination of UTP by CTP synthetase (Figure 22.22), with glutamine providing the nitrogen. [Note: Some CTP is dephosphorylated to cytidine diphosphate (CDP), which is a substrate for ribonucleotide reductase. The dCDP product can be phosphorylated to dCTP for DNA synthesis or dephosphoprylated to dCMP that is deaminated to dUMP.]

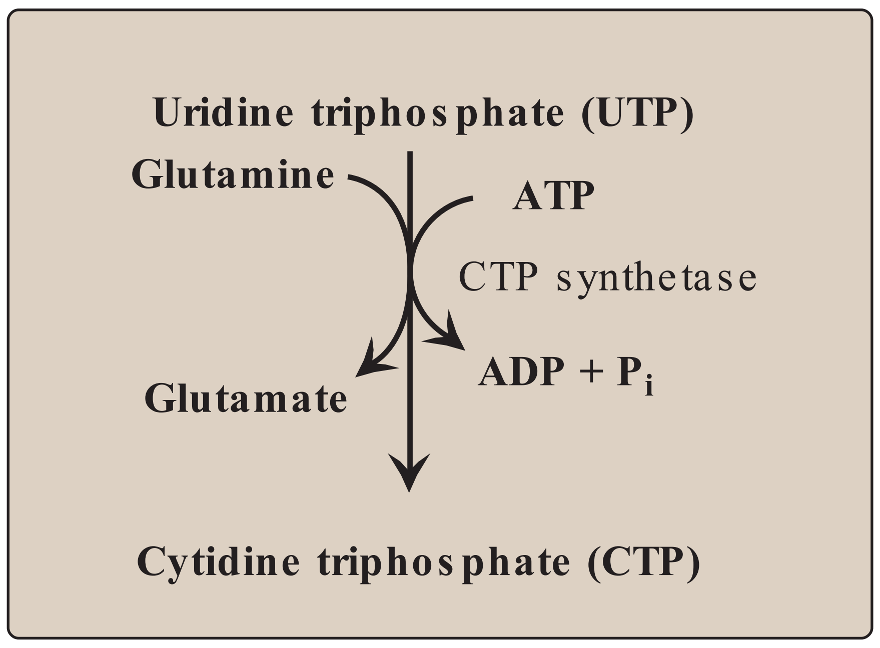

Uridine triphosphate (UTP)
Glutamine
ATP
CTP synthetase
Glutamate
ADP + Pi
Cytidine triphosphate (CTP)

Figure 22.22
Synthesis of CTP from UTP. [Note: CTP, required for RNA synthesis, is converted to dCTP for DNA synthesis.] ADP = adenosine diphosphate; P_i = inorganic phosphate.

E. Synthesis of deoxythymidine monophosphate

dUMP is converted to deoxythymidine monophosphate (dTMP) by thymidylate synthase, which uses N^5,N^{10}-methylene tetrahydrofolate as the source of the methyl group (see p. 267 for a discussion of this coenzyme). This is an unusual reaction in that tetrahydrofolate (THF) contributes not only a one-carbon unit but also two hydrogen atoms from the pteridine ring, resulting in the oxidation of THF to dihydrofolate ([DHF] Figure 22.23). Inhibitors of thymidylate synthase include thymine analogs such as 5-fluorouracil, which serve as antitumor agents. 5-Fluorouracil is metabolically converted to 5-fluorodeoxyuridine

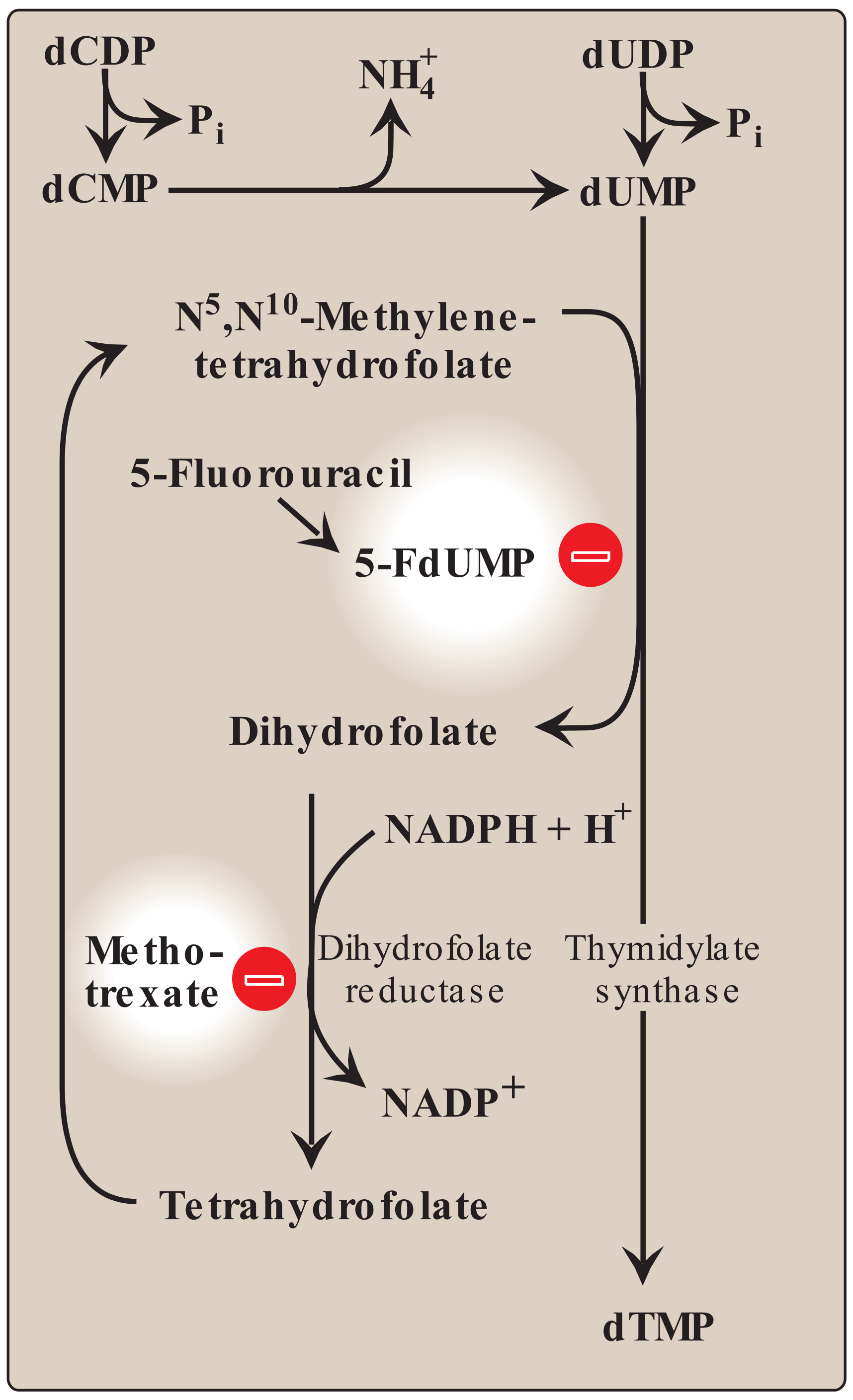


Figure 22.23
Synthesis of dTMP from dUMP, illustrating sites of action of antineoplastic drugs.

monophosphate (5-FdUMP), which becomes permanently bound to the inactivated thymidylate synthase, making the drug a "suicide" inhibitor (see p. 60). DHF can be reduced to THF by dihydrofolate reductase (see Figure 28.3, p. 374), an enzyme that is inhibited by folate analogs such as methotrexate. By decreasing the supply of THF, these drugs not only inhibit purine synthesis (see Figure 22.7), but, by preventing methylation of dUMP to dTMP, they also decrease the availability of this essential component of DNA. DNA synthesis is inhibited and cell growth slowed. Thus, these drugs are used to decrease the growth rate of cancer cells. [Note: Acyclovir (a purine analog) and AZT (3'-azido-3'-deoxythymidine, a pyrimidine analog) are used to treat infections of herpes simplex virus and human immunodeficiency virus, respectively. Each inhibits the viral DNA polymerase.]

F. Degradation and salvage of pyrimidines

Unlike the purine ring, which is not cleaved in humans, the pyrimidine ring is opened and degraded to highly soluble products, β-alanine (from the degradation of CMP and UMP) and β-aminoisobutyrate (from TMP degradation), with the production of NH_3 and CO_2. Pyrimidine bases can be salvaged to nucleosides, which are phosphorylated to nucleotides. However, their high solubility makes pyrimidine salvage less significant clinically than purine salvage. [Note: The salvage of pyrimidine nucleosides is the basis for using uridine in the treatment of hereditary orotic aciduria.]

VII. CHAPTER SUMMARY

Nucleotides are composed of a **nitrogenous base** (**adenine** = **A**, **guanine** = **G**, **cytosine** = **C**, **uracil** = **U**, and **thymine** = **T**); a **pentose**; and one, two, or three **phosphate groups** (Figure 22.24). A and G are **purines**, and C, U, and T are **pyrimidines**. If the sugar is **ribose**, the nucleotide is a **ribonucleoside phosphate** (for example, adenosine monophosphate [AMP]), and it can have several functions in the cell, including being a component of **RNA**. If the sugar is **deoxyribose**, the nucleotide is a **deoxyribonucleoside phosphate** (for example, deoxyAMP), and will be found almost exclusively as a component of **DNA**. The **committed step** in **purine synthesis** uses **5-phosphoribosyl-1-pyrophosphate** ([**PRPP**], an "activated pentose" that provides the ribose-phosphate group for de novo purine and pyrimidine synthesis and salvage) and nitrogen from **glutamine** to produce phosphoribosyl amine. The enzyme is glutamine:PRPP amidotransferase and is **inhibited** by **AMP** and **guanosine monophosphate** ([**GMP**] the end products of the pathway) and activated by PRPP. Purine nucleotides can also be produced from preformed purine bases by using **salvage reactions** catalyzed by adenine phosphoribosyltransferase (APRT) and hypoxanthine-guanine phosphoribosyltransferase (HGPRT). A near-total deficiency of HGPRT causes **Lesch-Nyhan syndrome**, a severe, heritable form of hyperuricemia accompanied by compulsive self-mutilation. All deoxyribonucleotides are synthesized from ribonucleotides by the enzyme ribonucleotide reductase. This enzyme is highly regulated (for example, it is **strongly inhibited by deoxyadenosine triphosphate** [**dATP**], a compound that is overproduced in bone marrow cells in individuals with adenosine deaminase **deficiency**). This syndrome causes **severe combined immunodeficiency disease**. The end product of purine degradation is **uric acid**, a compound whose overproduction or undersecretion causes **hyperuricemia** that, if accompanied by the deposition of monosodium urate crystals in joints and soft tissues and an inflammatory response to those crystals, results in **gout**. The first step in **pyrimidine synthesis**, the production of carbamoyl phosphate by carbamoyl phosphate synthetase II, is the **regulated** step in this pathway (it is **inhibited by uridine triphosphate** [**UTP**] and **activated by PRPP**). The UTP produced by this pathway can be converted to cytidine triphosphate. Deoxyuridine monophosphate can be converted to **deoxythymidine monophosphate** using thymidylate synthase, an enzyme targeted by anticancer drugs such as **5-fluorouracil**. The regeneration of tetrahydrofolate from dihydrofolate produced in the thymidylate synthase reaction requires dihydrofolate reductase, an enzyme targeted by the drug, **methotrexate**. Pyrimidine degradation results in soluble products.

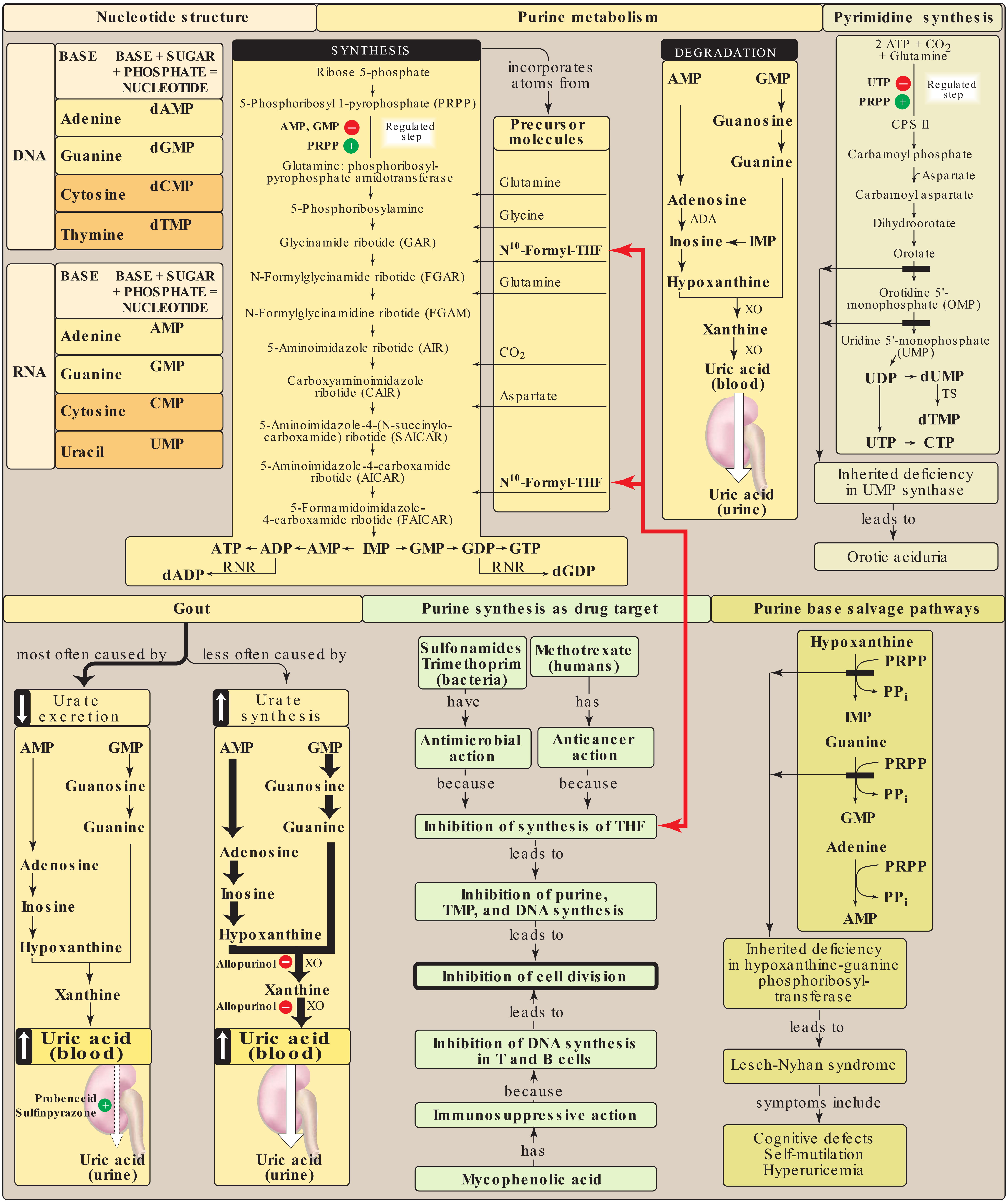

Nucleotide structure
DNA
BASE
BASE + SUGAR + PHOSPHATE = NUCLEOTIDE
Adenine dAMP
Guanine dGMP
Cytosine dCMP
Thymine dTMP
RNA
BASE
BASE + SUGAR + PHOSPHATE = NUCLEOTIDE
Adenine AMP
Guanine GMP
Cytosine CMP
Uracil UMP
Purine metabolism
SYNTHESIS
Ribose 5-phosphate
5-Phosphoribosyl 1-pyrophosphate (PRPP)
AMP, GMP
PRPP
Regulated step
Glutamine: phosphoribosylpyrophosphate amidotransferase
5-Phosphoribosylamine
Glycinamide ribotide (GAR)
N-Formylglycinamide ribotide (FGAR)
N-Formylglycinamidine ribotide (FGAM)
5-Aminoimidazole ribotide (AIR)
Carboxyaminoimidazole ribotide (CAIR)
5-Aminoimidazole-4-(N-succinylocarboxamide) ribotide (SAICAR)
5-Aminoimidazole-4-carboxamide ribotide (AICAR)
5-Formamidoimidazole-4-carboxamide ribotide (FAICAR)
ATP ← ADP ← AMP ← IMP → GMP → GDP → GTP
dADP ← RNR
RNR → dGDP
incorporates atoms from
Precursor molecules
Glutamine
Glycine
N^{10}-Formyl-THF
Glutamine
CO$_2$
Aspartate
N^{10}-Formyl-THF
DEGRADATION
AMP
GMP
Guanosine
Guanine
Adenosine
ADA
Inosine ← IMP
Hypoxanthine
XO
Xanthine
XO
Uric acid (blood)
Uric acid (urine)
Pyrimidine synthesis
2 ATP + CO$_2$ + Glutamine
UTP
PRPP
Regulated step
CPS II
Carbamoyl phosphate
Aspartate
Carbamoyl aspartate
Dihydroorotate
Orotate
Orotidine 5'-monophosphate (OMP)
Uridine 5'-monophosphate (UMP)
UDP → dUMP
TS
dTMP
UTP → CTP
Inherited deficiency in UMP synthase
leads to
Orotic aciduria
Gout
most often caused by
less often caused by
Urate excretion
AMP
GMP
Guanosine
Guanine
Adenosine
Inosine
Hypoxanthine
Xanthine
Uric acid (blood)
Probenecid
Sulfinpyrazone
Uric acid (urine)
Urate synthesis
AMP
GMP
Guanosine
Guanine
Adenosine
Inosine
Hypoxanthine
Allopurinol
XO
Xanthine
Allopurinol
XO
Uric acid (blood)
Uric acid (urine)
Purine synthesis as drug target
Sulfonamides Trimethoprim (bacteria)
Methotrexate (humans)
have
has
Antimicrobial action
Anticancer action
because
because
Inhibition of synthesis of THF
leads to
Inhibition of purine, TMP, and DNA synthesis
leads to
Inhibition of cell division
leads to
Inhibition of DNA synthesis in T and B cells
because
Immunosuppressive action
has
Mycophenolic acid
Purine base salvage pathways
Hypoxanthine
PRPP
PP$_i$
IMP
Guanine
PRPP
PP$_i$
GMP
Adenine
PRPP
PP$_i$
AMP
Inherited deficiency in hypoxanthine-guanine phosphoribosyltransferase
leads to
Lesch-Nyhan syndrome
symptoms include
Cognitive defects
Self-mutilation
Hyperuricemia

Figure 22.24

Key concept map for nucleotide metabolism. THF = tetrahydrofolate; ADA= adenosine deaminase; XO = xanthine oxidase; TS = thymidylate synthase; RNR = ribonucleotide reductase; CPS II = carbamyl phosphate synthetase II; AMP = adenosine monophosphate; GMP = guanosine monophosphate; CMP = cytidine monophosphate; TMP = thymidine monophosphate; IMP = inosine monophosphate; d = deoxy; PP$_i$ = pyrophosphate.

UNIT V:
Integration of Metabolism

Metabolic Effects of Insulin and Glucagon

23

I. OVERVIEW

Four major tissues play a dominant role in fuel metabolism: liver, adipose, muscle, and brain. These tissues contain unique sets of enzymes, such that each tissue is specialized for the storage, use, or generation of specific fuels. These tissues do not function in isolation, but rather form part of a network in which one tissue may provide substrates to another or process compounds produced by other organs. Communication between tissues is mediated by the nervous system, by the availability of circulating substrates, and by variation in the levels of plasma hormones (Figure 23.1). The integration of energy metabolism is controlled primarily by the actions of two peptide hormones, insulin and glucagon (secreted in response to changing substrate levels in the blood), with the catecholamines epinephrine and norepinephrine (secreted in response to neural signals) playing a supporting role. Changes in the circulating levels of these hormones allow the body to store energy when food is abundant or to make stored energy available such as during "survival crises" (for example, famine, severe injury, and "fight-or-flight" situations). This chapter describes the structure, secretion, and metabolic effects of the two hormones that most profoundly affect energy metabolism.

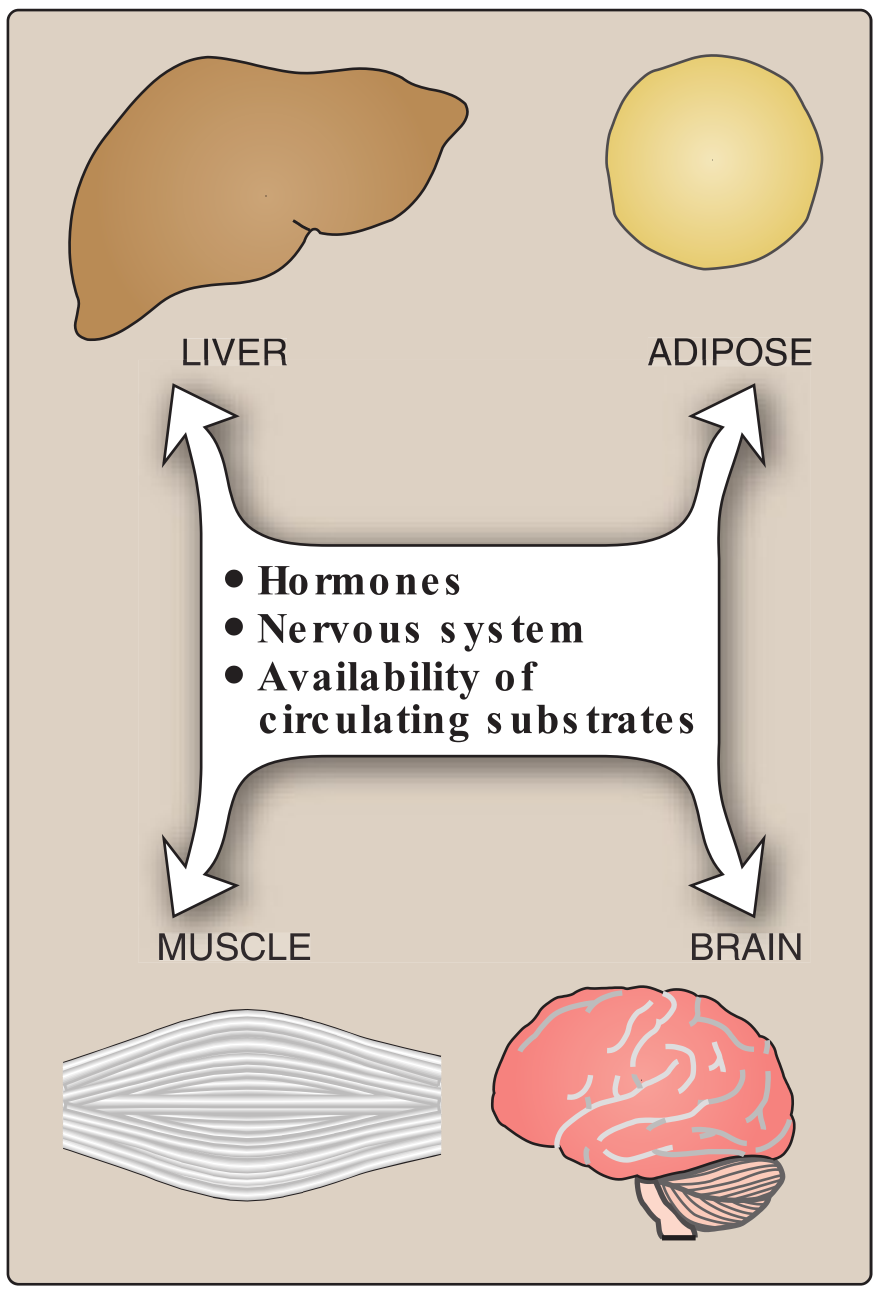


Figure 23.1
Mechanisms of communication between four major tissues.

II. INSULIN

Insulin is a peptide hormone produced by the β cells of the islets of Langerhans, which are clusters of cells that are embedded in the endocrine portion of the pancreas (Figure 23.2). [Note: "Insulin" is from the Latin for island.] The islets make up only about 1%–2% of the total cells of the pancreas. Insulin is the most important hormone coordinating the

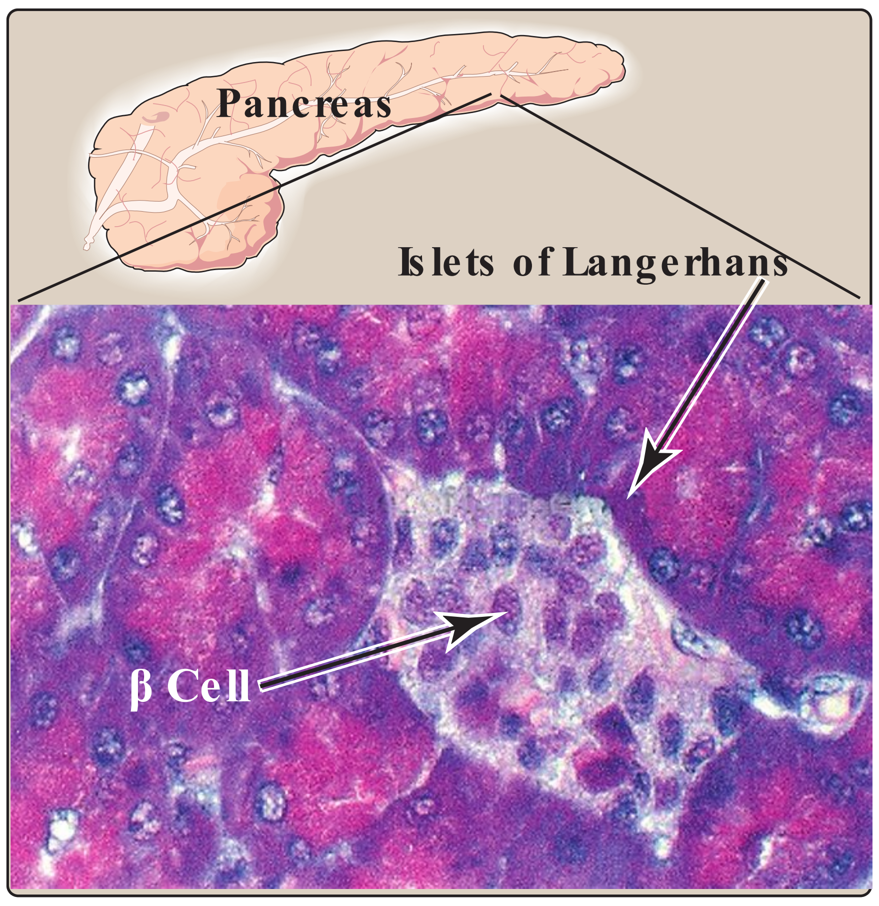

Pancreas
Islets of Langerhans
β Cell

Figure 23.2
Islets of Langerhans.

use of fuels by tissues. Its metabolic effects are anabolic, favoring, for example, synthesis of glycogen, triacylglycerols (TAGs), and protein.

A. Structure of insulin

Insulin is composed of 51 amino acids arranged in two polypeptide chains, designated A (21 amino acids) and B, which are linked together by two disulfide bridges (Figure 23.3A). The insulin molecule also contains an intramolecular disulfide bridge between amino acid residues of the A chain. [Note: Insulin was the first peptide for which the primary structure was determined, and the first therapeutic molecule made by recombinant DNA technology (see p. 470).]

B. Synthesis of insulin

The processing and transport of intermediates that occur during the synthesis of insulin are shown in Figures 23.3B and 23.4. Biosynthesis involves production of two inactive precursors, preproinsulin and proinsulin, that are sequentially cleaved to form the active hormone plus the connecting or C-peptide (see Figure 23.4). [Note: The C-peptide is essential for proper insulin folding. Also, because its half-life in the plasma is longer than that of insulin, the C-peptide is a good indicator of insulin production and secretion.] Insulin is stored in the cytosol in granules that, given the proper stimulus (see below), are released by exocytosis. (See p. 166 for a discussion of the synthesis of secreted proteins.) Insulin is degraded by insulin-degrading enzyme, which is present in the liver and, to a lesser extent, in the kidneys. Insulin has a plasma half-life of approximately 6 minutes. This short duration of action permits rapid changes in circulating levels of the hormone.

C. Regulation of insulin secretion

Secretion of insulin is regulated by bloodborne fuels and hormones.

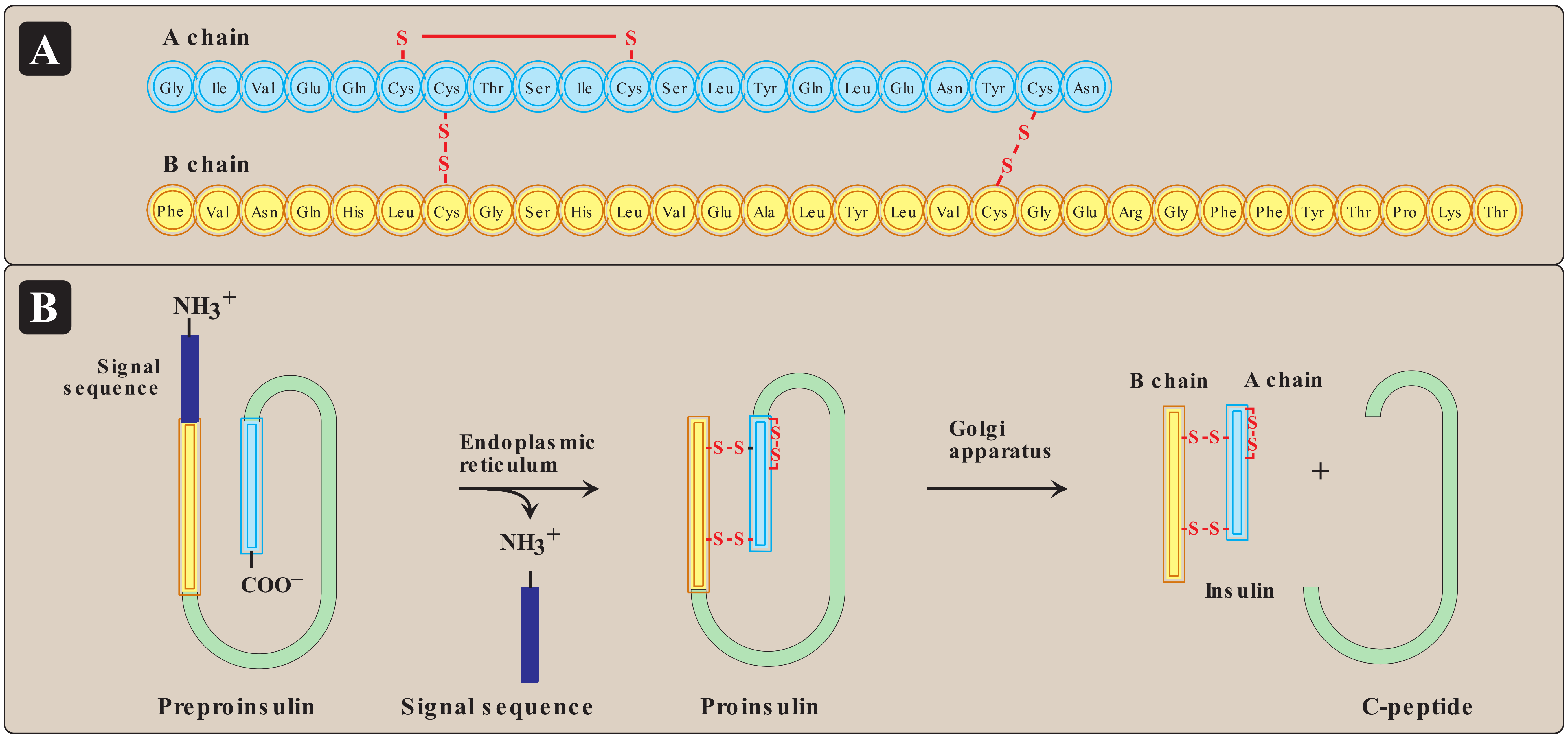

A
A chain
S
S
Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
S
S
S
S
B chain
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr
B
NH3+
Signal sequence
COO−
Endoplasmic reticulum
NH3+
S-S
S-S
S-S
Golgi apparatus
B chain
A chain
S-S
S-S
S-S
+
Insulin
Preproinsulin
Signal sequence
Proinsulin
C-peptide

Figure 23.3
A. Structure of insulin. B. Formation of human insulin from preproinsulin.

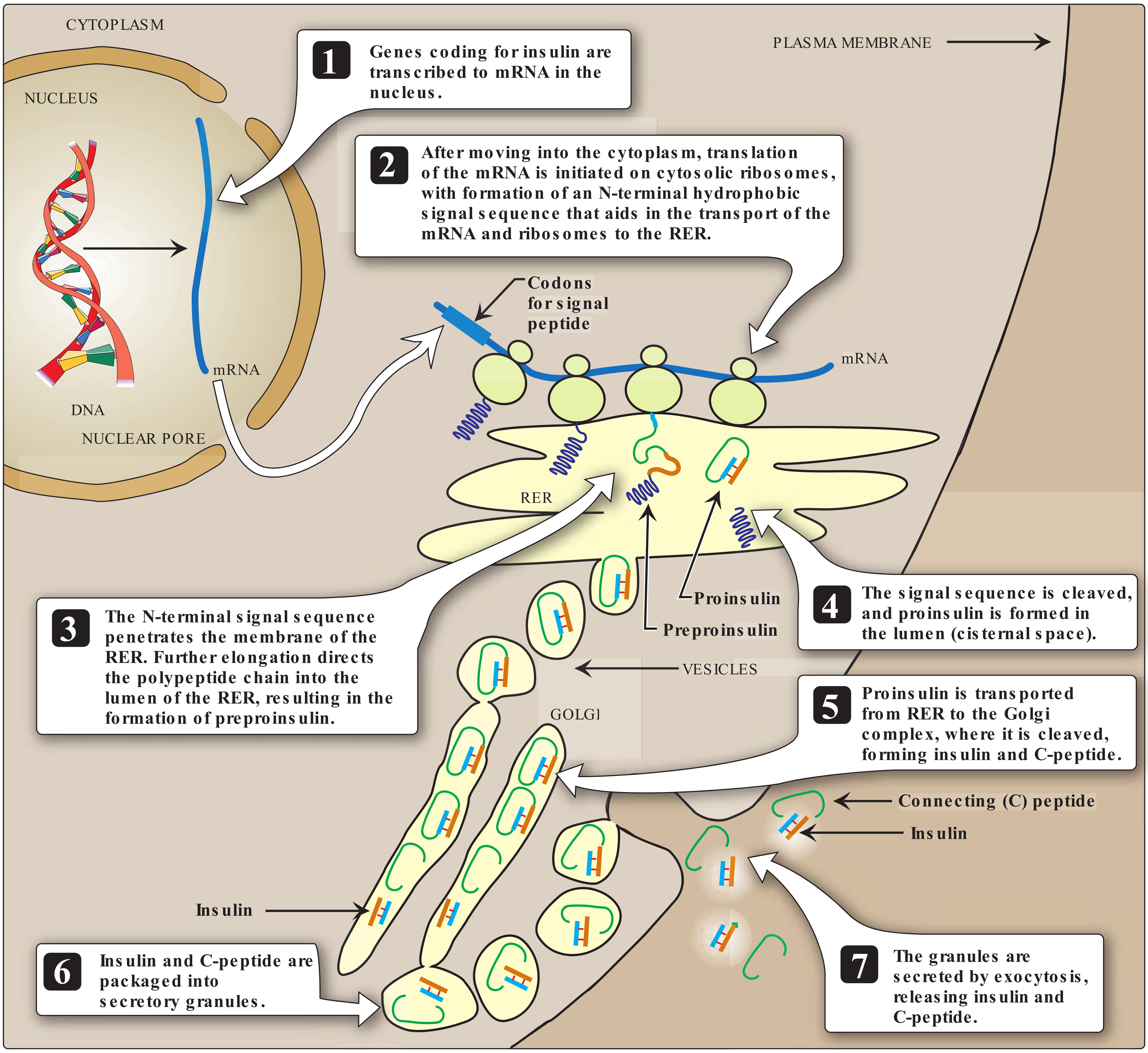

CYTOPLASM
NUCLEUS
DNA
mRNA
NUCLEAR PORE
PLASMA MEMBRANE
Codons for signal peptide
mRNA
RER
Proinsulin
Preproinsulin
VESICLES
GOLGI
Connecting (C) peptide
Insulin
Insulin
1 Genes coding for insulin are transcribed to mRNA in the nucleus.
2 After moving into the cytoplasm, translation of the mRNA is initiated on cytosolic ribosomes, with formation of an N-terminal hydrophobic signal sequence that aids in the transport of the mRNA and ribosomes to the RER.
3 The N-terminal signal sequence penetrates the membrane of the RER. Further elongation directs the polypeptide chain into the lumen of the RER, resulting in the formation of preproinsulin.
4 The signal sequence is cleaved, and proinsulin is formed in the lumen (cisternal space).
5 Proinsulin is transported from RER to the Golgi complex, where it is cleaved, forming insulin and C-peptide.
6 Insulin and C-peptide are packaged into secretory granules.
7 The granules are secreted by exocytosis, releasing insulin and C-peptide.

Figure 23.4
Intracellular movements of insulin and its precursors. mRNA = messenger RNA; RER = rough endoplasmic reticulum.

1. **Stimulation of insulin secretion:** Insulin secretion by the pancreatic β cells is closely coordinated with the release of glucagon by pancreatic α cells (Figure 23.5). The relative amounts of insulin and glucagon released by the pancreas are regulated so that the rate of hepatic glucose production is kept equal to the use of glucose by peripheral tissues. In view of its coordinating role, it is not surprising that the β cell responds to a variety of stimuli. In particular, insulin secretion is increased by glucose, amino acids, and gastrointestinal peptide hormones.

   a. **Glucose:** Ingestion of a carbohydrate-rich meal leads to a rise in blood glucose, the primary stimulus for insulin secretion (see Figure 23.5). The β cells are the most important glucose-sensing

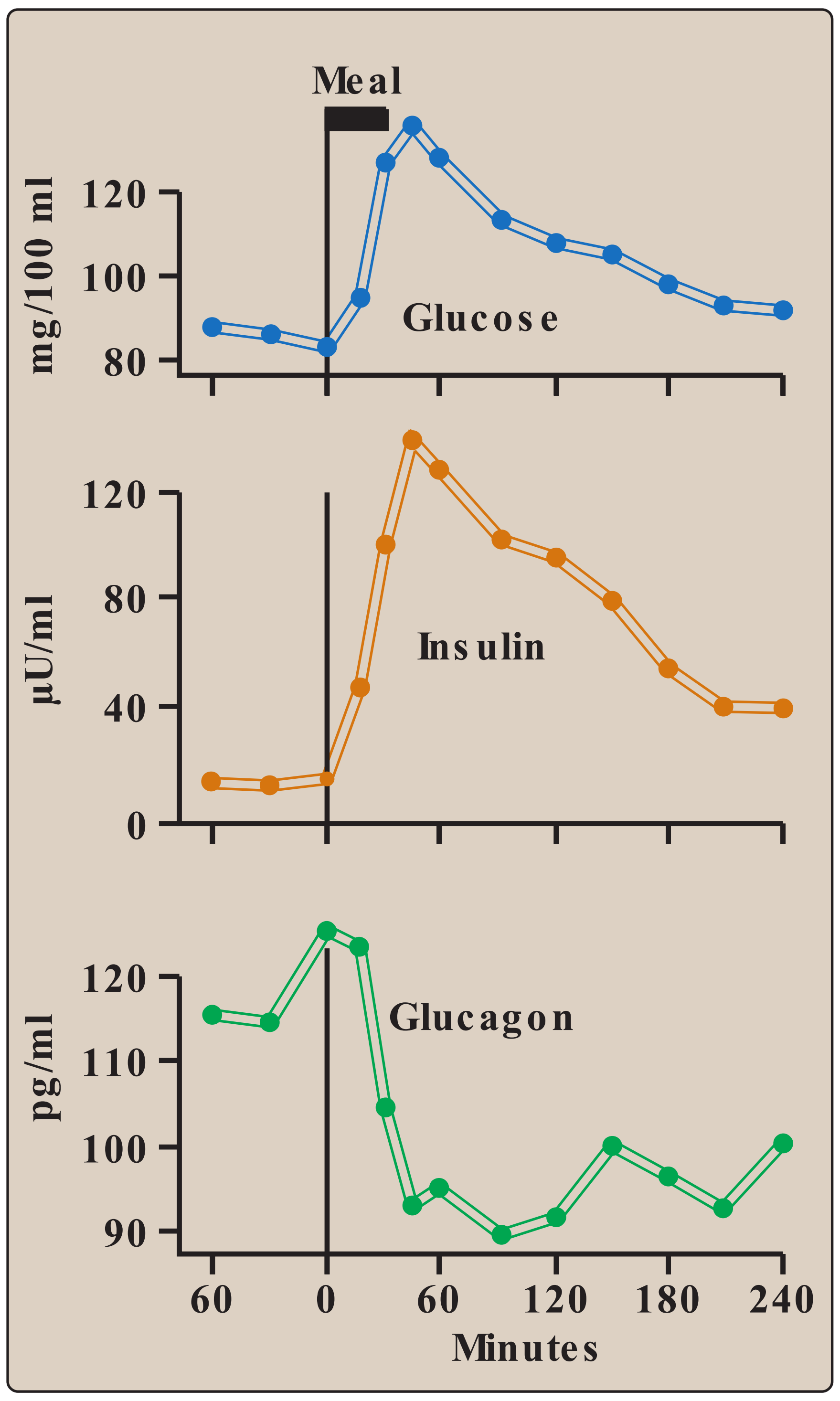


Figure 23.5
Changes in blood levels of glucose, insulin, and glucagon after ingestion of a carbohydrate-rich meal.

cells in the body. Like the liver, β cells contain GLUT-2 transporters and express glucokinase (hexokinase IV; see p. 98). At blood glucose levels above 45 mg/dl, glucokinase phosphorylates glucose in amounts proportional to the glucose concentration. Proportionality results from the lack of direct inhibition of glucokinase by glucose 6-phosphate, its product. Additionally, the sigmoidal relationship between the velocity of the reaction and substrate concentration (see p. 98) maximizes the enzyme's responsiveness to changes in blood glucose level. Metabolism of glucose 6-phosphate generates adenosine triphosphate (ATP), leading to insulin secretion (see blue box below).

b. **Amino acids:** Ingestion of protein causes a transient rise in plasma amino acid levels (for example, arginine) that enhances the glucose-stimulated secretion of insulin. [Note: Fatty acids have a similar effect.]

c. **Gastrointestinal peptide hormones:** The intestinal peptides glucagon-like protein-1 (GLP-1) and gastric-inhibitory polypeptide ([GIP]; also called glucose-dependent insulinotropic peptide) increase the sensitivity of β cells to glucose. They are released from the small intestine after the ingestion of food, causing an anticipatory rise in insulin levels and, thus, are referred to as "incretins." Their action may account for the fact that the same amount of glucose given orally induces a much greater secretion of insulin than if given intravenously (IV).

Glucose-dependent release of insulin into blood is mediated through a rise in calcium (Ca^{2+}) concentration in the β cell. Glucose taken into β cells is phosphorylated and metabolized, with subsequent production of ATP. ATP-sensitive potassium (K^+) channels close, causing depolarization of the plasma membrane, opening of voltage-gated Ca^{2+} channels, and influx of Ca^{2+} into the cell. Ca^{2+} causes vesicles containing insulin to be exocytosed from the β cell. Sulfonylureas, oral agents used to treat type 2 diabetes, increase insulin secretion by closing ATP-sensitive K^+ channels.

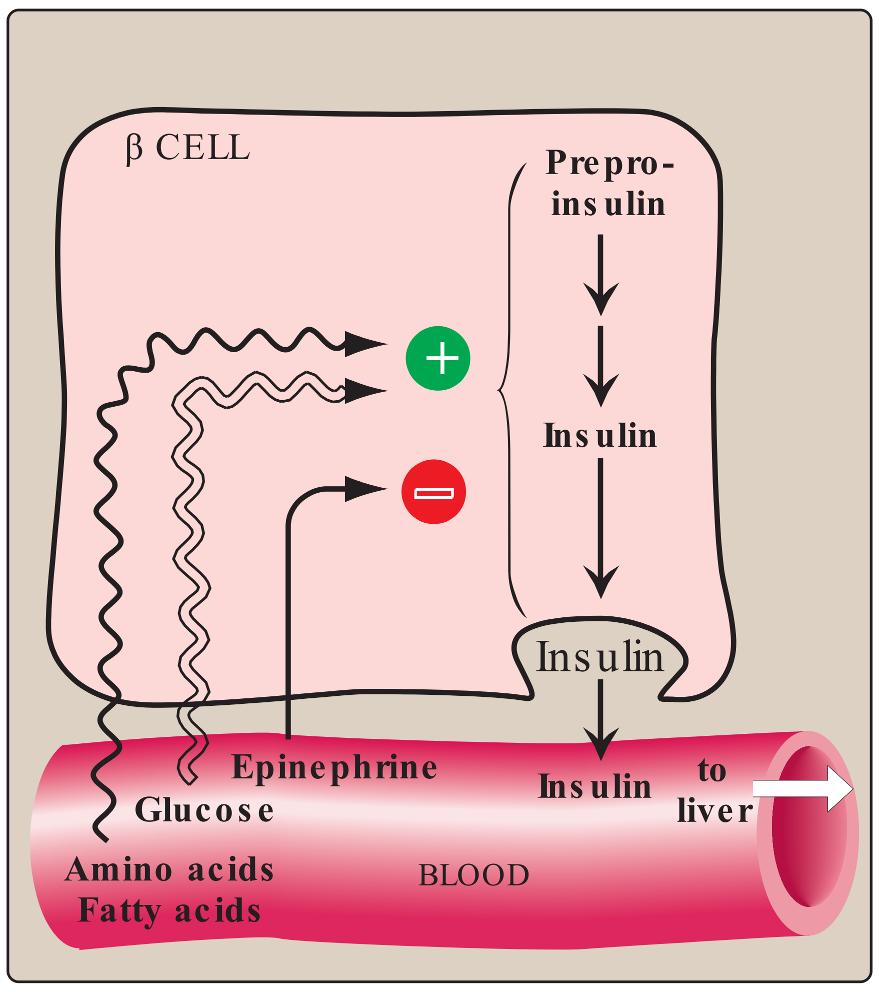


Figure 23.6
Regulation of insulin release from pancreatic β cells. [Note: Gastrointestinal peptide hormones also stimulate insulin release.]

2. **Inhibition of insulin secretion:** The synthesis and release of insulin are decreased when there is a scarcity of dietary fuels and also during periods of physiologic stress (for example, infection, hypoxia, and vigorous exercise). These effects are mediated primarily by the catecholamines norepinephrine and epinephrine, which are made from tyrosine in the sympathetic nervous system and the adrenal medulla and secreted. Secretion is largely controlled by the nervous system. The catecholamines (primarily epinephrine) have a direct effect on energy metabolism, causing a rapid mobilization of energy-yielding fuels, including glucose from the liver (produced by glycogenolysis or gluconeogenesis; see p. 121) and fatty acids from adipose tissue (produced by lipolysis; see p. 189). In addition, these biogenic amines can override the normal glucose-stimulated release of insulin. Thus, in emergency

situations, the sympathetic nervous system largely replaces the plasma glucose concentration as the controlling influence over β-cell secretion. The regulation of insulin secretion is summarized in Figure 23.6.

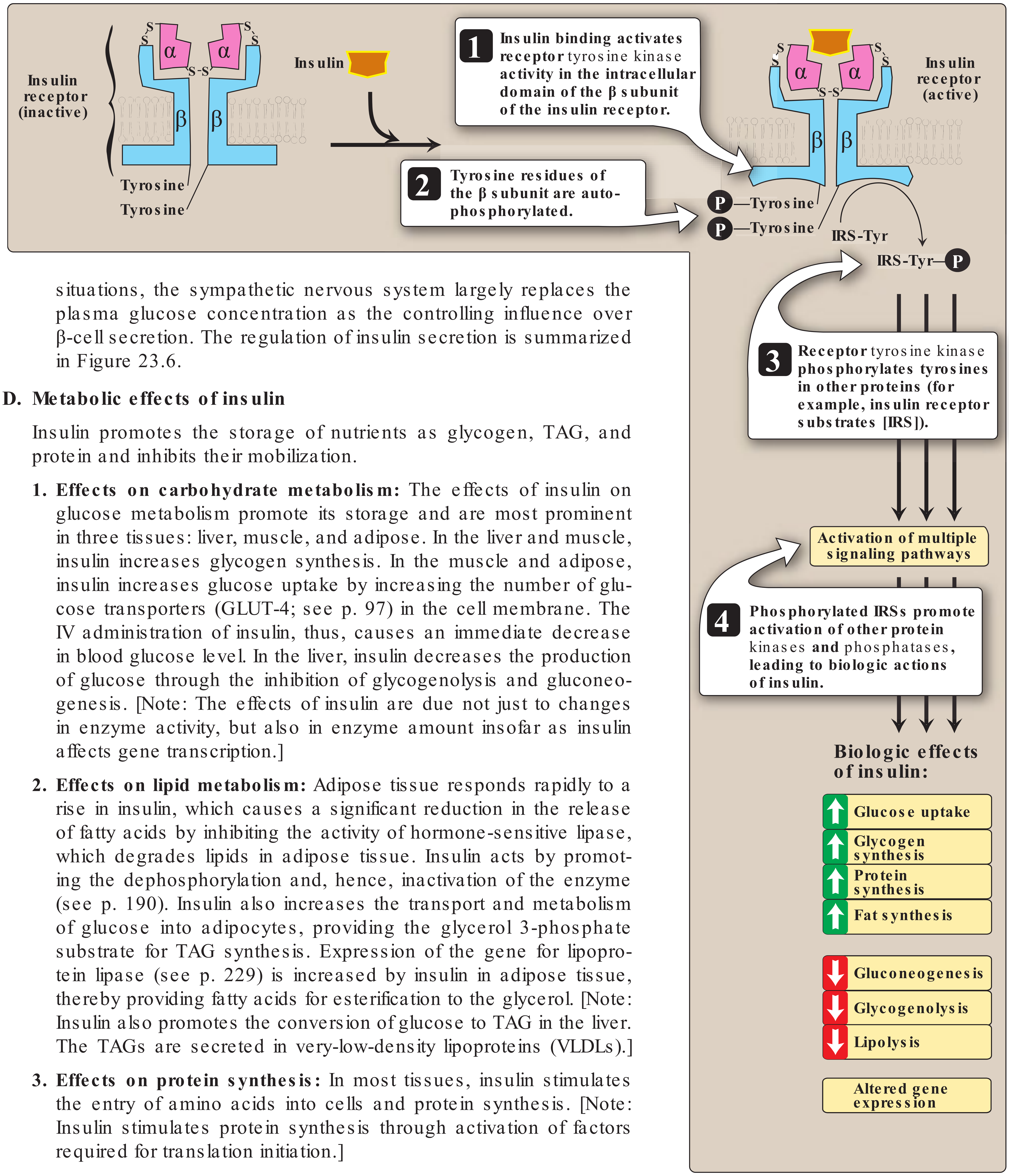
D. Metabolic effects of insulin

Insulin promotes the storage of nutrients as glycogen, TAG, and protein and inhibits their mobilization.

1. **Effects on carbohydrate metabolism:** The effects of insulin on glucose metabolism promote its storage and are most prominent in three tissues: liver, muscle, and adipose. In the liver and muscle, insulin increases glycogen synthesis. In the muscle and adipose, insulin increases glucose uptake by increasing the number of glucose transporters (GLUT-4; see p. 97) in the cell membrane. The IV administration of insulin, thus, causes an immediate decrease in blood glucose level. In the liver, insulin decreases the production of glucose through the inhibition of glycogenolysis and gluconeogenesis. [Note: The effects of insulin are due not just to changes in enzyme activity, but also in enzyme amount insofar as insulin affects gene transcription.]
2. **Effects on lipid metabolism:** Adipose tissue responds rapidly to a rise in insulin, which causes a significant reduction in the release of fatty acids by inhibiting the activity of hormone-sensitive lipase, which degrades lipids in adipose tissue. Insulin acts by promoting the dephosphorylation and, hence, inactivation of the enzyme (see p. 190). Insulin also increases the transport and metabolism of glucose into adipocytes, providing the glycerol 3-phosphate substrate for TAG synthesis. Expression of the gene for lipoprotein lipase (see p. 229) is increased by insulin in adipose tissue, thereby providing fatty acids for esterification to the glycerol. [Note: Insulin also promotes the conversion of glucose to TAG in the liver. The TAGs are secreted in very-low-density lipoproteins (VLDLs).]
3. **Effects on protein synthesis:** In most tissues, insulin stimulates the entry of amino acids into cells and protein synthesis. [Note: Insulin stimulates protein synthesis through activation of factors required for translation initiation.]

E. Mechanism of insulin action

Insulin binds to specific, high-affinity receptors in the cell membrane of most tissues, including liver, muscle, and adipose. This is the first step in a cascade of reactions ultimately leading to a diverse array of biologic actions (Figure 23.7).


Insulin receptor (inactive)
S S
α α
S-S
β β
Tyrosine
Tyrosine
Insulin
Insulin receptor (active)
P—Tyrosine
P—Tyrosine
IRS-Tyr
IRS-Tyr—P
Activation of multiple signaling pathways
Biologic effects of insulin:
↑ Glucose uptake
↑ Glycogen synthesis
↑ Protein synthesis
↑ Fat synthesis
↓ Gluconeogenesis
↓ Glycogenolysis
↓ Lipolysis
Altered gene expression

1 **Insulin binding activates receptor** tyrosine kinase **activity in the intracellular domain of the β subunit of the insulin receptor.**

2 **Tyrosine residues of the β subunit are autophosphorylated.**

3 **Receptor** tyrosine kinase **phosphorylates tyrosines in other proteins (for example, insulin receptor substrates [IRS]).**

4 **Phosphorylated IRSs promote activation of other protein** kinases **and** phosphatases, **leading to biologic actions of insulin.**

Figure 23.7
Insulin receptor. P = phosphate; Tyr = tyrosine.

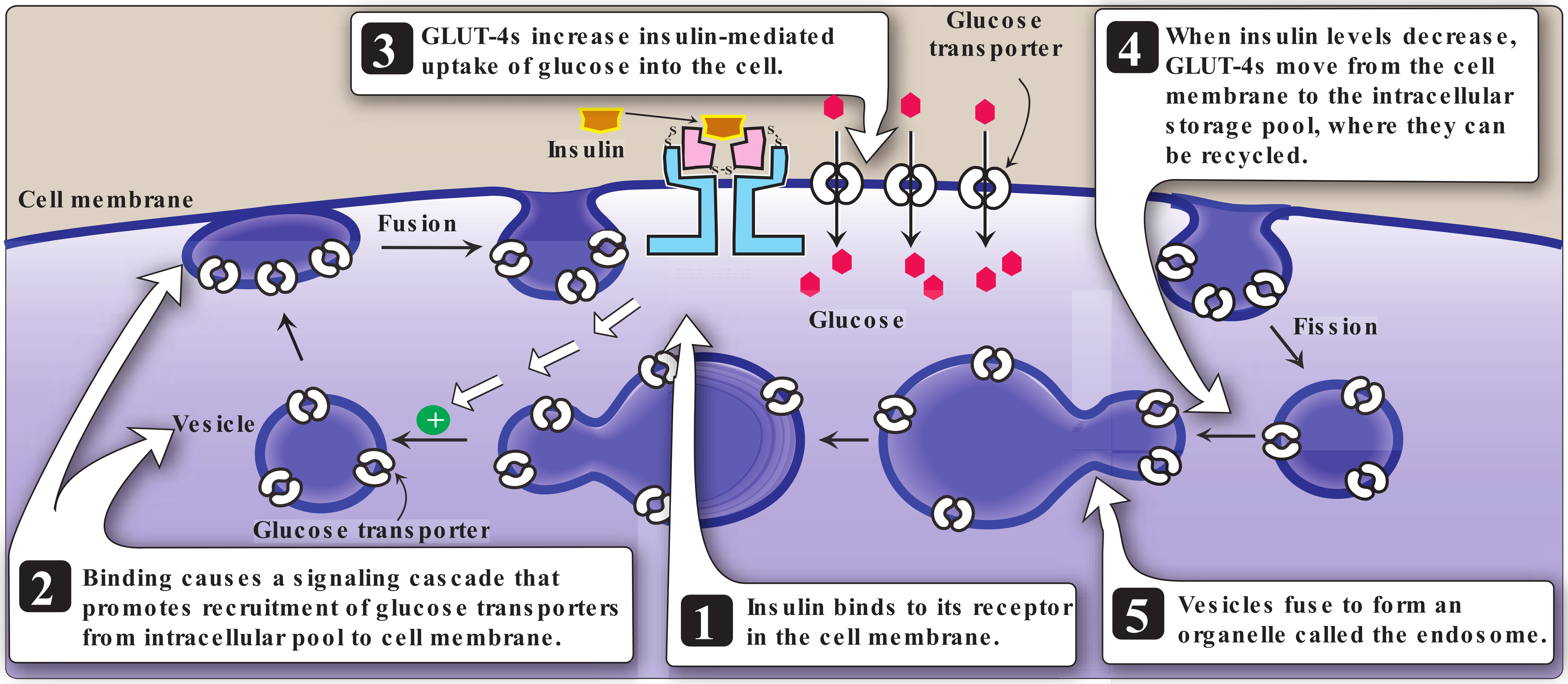

3 GLUT-4s increase insulin-mediated uptake of glucose into the cell.
Glucose transporter
4 When insulin levels decrease, GLUT-4s move from the cell membrane to the intracellular storage pool, where they can be recycled.
Insulin
S-S
Cell membrane
Fusion
Glucose
Fission
Vesicle
+
Glucose transporter
2 Binding causes a signaling cascade that promotes recruitment of glucose transporters from intracellular pool to cell membrane.
1 Insulin binds to its receptor in the cell membrane.
5 Vesicles fuse to form an organelle called the endosome.

Figure 23.8
Insulin-mediated recruitment of glucose transporters (GLUT-4s) from intracellular stores in skeletal and cardiac muscle and adipose tissue.

	Active transport	Facilitated transport
Insulin sensitive		Skeletal and cardiac muscle and adipose tissue (together account for largest tissue mass)
Insulin insensitive	Epithelia of intestine Renal tubules Choroid plexus	Erythrocytes Leukocytes Lens of eye Cornea Liver Brain

Figure 23.9
Characteristics of glucose transport in various tissues.

1. **Insulin receptor:** The insulin receptor is synthesized as a single polypeptide that is glycosylated and cleaved into α and β subunits, which are then assembled into a tetramer linked by disulfide bonds (see Figure 23.7). The extracellular α subunit contains the insulin-binding site. A hydrophobic domain in each β subunit spans the plasma membrane. The cytosolic domain of the β subunit is a tyrosine kinase, which is activated by insulin. As a result, the insulin receptor is classified as a tyrosine-kinase receptor.

2. **Signal transduction:** The binding of insulin to the α subunits of the insulin receptor induces conformational changes that are transmitted to the β subunits. This promotes a rapid autophosphorylation of specific tyrosine residues on each β subunit (see Figure 23.7). Autophosphorylation initiates a cascade of cell-signaling responses, including phosphorylation of a family of proteins called insulin receptor substrates (IRSs). At least four IRSs have been identified that show similar structures but different tissue distributions. Phosphorylated IRS proteins interact with other signaling molecules through specific domains, activating a number of pathways that affect gene expression, cell metabolism, and growth. The actions of insulin are terminated by dephosphorylation of the receptor.

3. **Membrane effects of insulin:** Glucose transport in some tissues, such as muscle and adipose, increases in the presence of insulin (Figure 23.8). Insulin promotes movement of insulin-sensitive glucose transporters (GLUT-4s) from a pool located in intracellular vesicles to the cell membrane. [Note: Movement is the result of a signaling cascade in which an IRS binds to and activates a kinase (phosphoinositide 3-kinase), leading to phosphorylation of the membrane phospholipid phosphatidylinositol 4,5-bisphosphate to the 3,4,5-trisphosphate form that binds to and activates phosphoinositide kinase 1. This kinase, in turn, activates Akt (protein kinase B), resulting in GLUT-4 movement.] In contrast, other

tissues have insulin-insensitive systems for glucose transport (Figure 23.9). For example, hepatocytes; erythrocytes; and cells of the nervous system, intestinal mucosa, renal tubules, and cornea do not require insulin for glucose uptake.

4. **Receptor regulation:** Binding of insulin is followed by internalization of the hormone–receptor complex. Once inside the cell, insulin is degraded in the lysosomes. The receptors may be degraded, but most are recycled to the cell surface. [Note: Elevated levels of insulin promote the degradation of receptors, therby decreasing the number of surface receptors. This is one type of "downregulation."]

5. **Time course of insulin actions:** The binding of insulin provokes a wide range of actions. The most immediate response is an increase in glucose transport into adipocytes and skeletal and cardiac muscle cells that occurs within seconds of insulin binding to its membrane receptor. Insulin-induced changes in enzymic activity in many cell types occur over minutes to hours and reflect changes in the phosphorylation states of existing proteins. Insulin-induced increase in the amount of many enzymes, such as glucokinase, liver pyruvate kinase, acetyl coenzyme A (CoA) carboxylase (ACC), and fatty acid synthase, requires hours to days. These changes reflect an increase in gene expression through increased transcription (mediated by sterol regulatory element–binding protein-1; see p. 184) and translation.

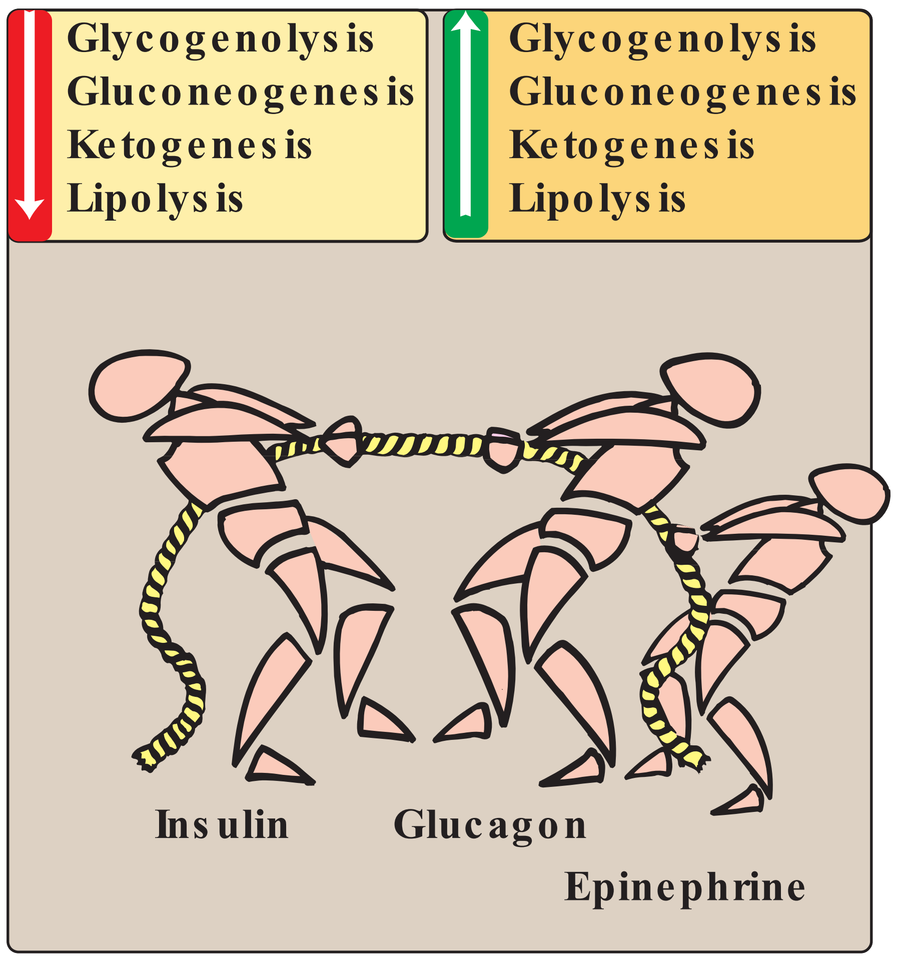


Figure 23.10
Opposing actions of insulin and glucagon plus epinephrine.

III. GLUCAGON

Glucagon is a peptide hormone secreted by the α cells of the pancreatic islets of Langerhans. Glucagon, along with epinephrine, norepinephrine, cortisol, and growth hormone (the "counterregulatory" hormones), opposes many of the actions of insulin (Figure 23.10). Most importantly, glucagon acts to maintain blood glucose levels by activation of hepatic glycogenolysis and gluconeogenesis. Glucagon is composed of 29 amino acids arranged in a single polypeptide chain. [Note: Unlike insulin, the amino acid sequence of glucagon is the same in all mammalian species examined to date.] Glucagon is synthesized as a large precursor molecule (preproglucagon) that is converted to glucagon through a series of selective proteolytic cleavages, similar to those described for insulin biosynthesis (see Figure 23.3). In contrast to insulin, preproglucagon is processed to different products in different tissues, for example, GLP-1 in intestinal L cells. Like insulin, glucagon has a short half-life.

A. Stimulation of glucagon secretion

The α cell is responsive to a variety of stimuli that signal actual or potential hypoglycemia (Figure 23.11). Specifically, glucagon secretion is increased by low blood glucose, amino acids, and catecholamines.

1. **Low blood glucose:** A decrease in plasma glucose concentration is the primary stimulus for glucagon release. During an overnight or prolonged fast, elevated glucagon levels prevent hypoglycemia (see below for a discussion of hypoglycemia).

2. **Amino acids:** Amino acids (for example, arginine) derived from a meal containing protein stimulate the release of glucagon. The glucagon effectively prevents the hypoglycemia that would otherwise occur as a result of the increased insulin secretion that also occurs after a protein meal.

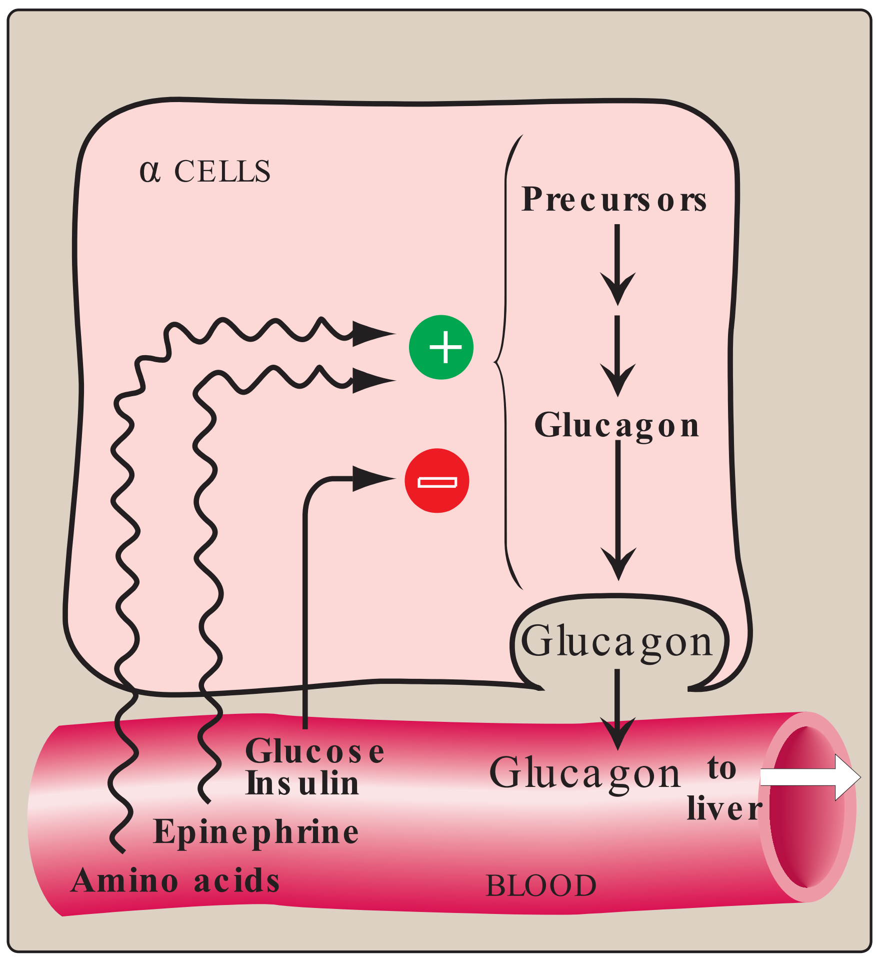


Figure 23.11
Regulation of glucagon release from pancreatic α cells. [Note: Amino acids increase release of insulin and glucagon, whereas glucose increases release of insulin only.]

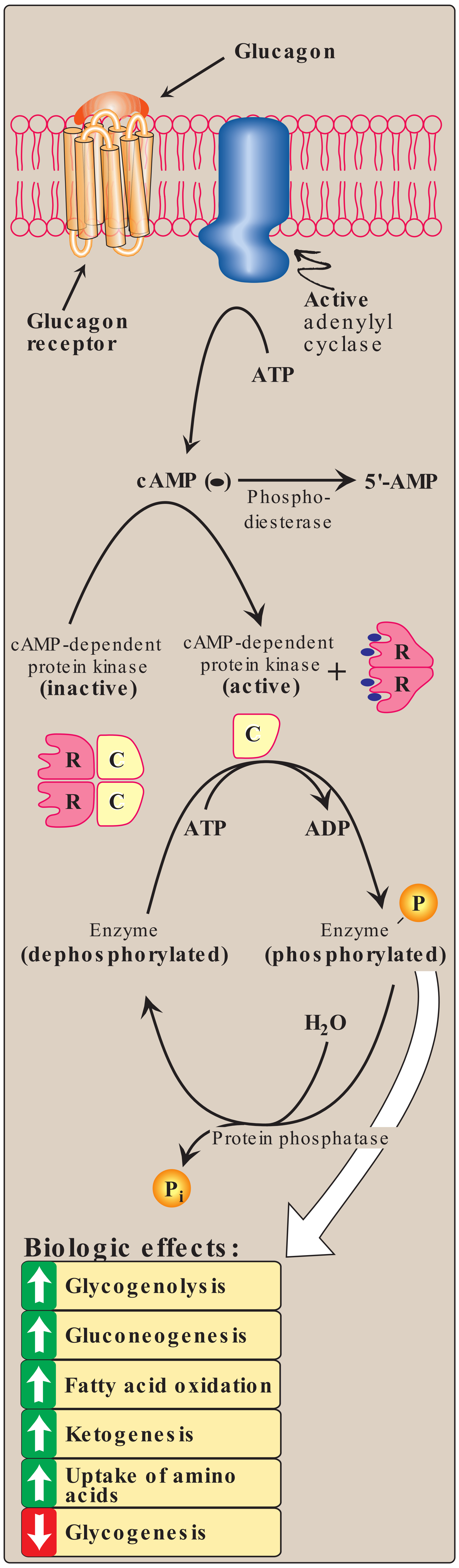

Glucagon
Glucagon receptor
Active adenylyl cyclase
ATP
cAMP (●)
Phosphodiesterase
5'-AMP
cAMP-dependent protein kinase (inactive)
cAMP-dependent protein kinase (active)
+
R
R
C
C
R
R
C
ATP
ADP
P
Enzyme (dephosphorylated)
Enzyme (phosphorylated)
H_2O
Protein phosphatase
P_i
Biologic effects:
Glycogenolysis
Gluconeogenesis
Fatty acid oxidation
Ketogenesis
Uptake of amino acids
Glycogenesis

Figure 23.12
Mechanism of action of glucagon. [Note: For clarity, G-protein activation of adenylyl cyclase has been omitted.] R = regulatory subunit; C = catalytic subunit; cAMP = cyclic adenosine monophosphate; ADP = adenosine diphosphate; P = phosphate.

3. **Catecholamines:** Elevated levels of circulating epinephrine produced by the adrenal medulla, norepinephrine produced by sympathetic innervation of the pancreas, or both stimulate the release of glucagon. Thus, during periods of physiologic stress, the elevated catecholamine levels can override the effect on the α cell of circulating substrates. In these situations, regardless of the concentration of blood glucose, glucagon levels are elevated in anticipation of increased glucose use. In contrast, insulin levels are depressed.

B. Inhibition of glucagon secretion

Glucagon secretion is significantly decreased by elevated blood glucose and by insulin. Both substances are increased following ingestion of glucose or a carbohydrate-rich meal (see Figure 23.5). The regulation of glucagon secretion is summarized in Figure 23.11.

C. Metabolic effects of glucagon

Glucagon is a catabolic hormone that promotes the maintenance of blood glucose levels. Its primary target is the liver.

1. **Effects on carbohydrate metabolism:** The IV administration of glucagon leads to an immediate rise in blood glucose. This results from an increase in the breakdown of liver glycogen and an increase in hepatic gluconeogenesis.

2. **Effects on lipid metabolism:** The primary effect of glucagon on lipid metabolism is inhibition of fatty acid synthesis through phosphorylation of ACC (see p. 184). The decrease in malonyl CoA production as a result of ACC inhibition removes the break on long-chain fatty acid β-oxidation. Glucagon also plays a role in lipolysis in adipose, but the major activators of hormone sensitive lipase (via phosphorylation by protein kinase A) are the catecholamines. The free fatty acids released are taken up by liver and oxidized to acetyl CoA, which is used in ketone body synthesis.

3. **Effects on protein metabolism:** Glucagon increases uptake by the liver of amino acids supplied by muscle, resulting in increased availability of carbon skeletons for gluconeogenesis. As a consequence, plasma levels of amino acids are decreased.

D. Mechanism of action of glucagon

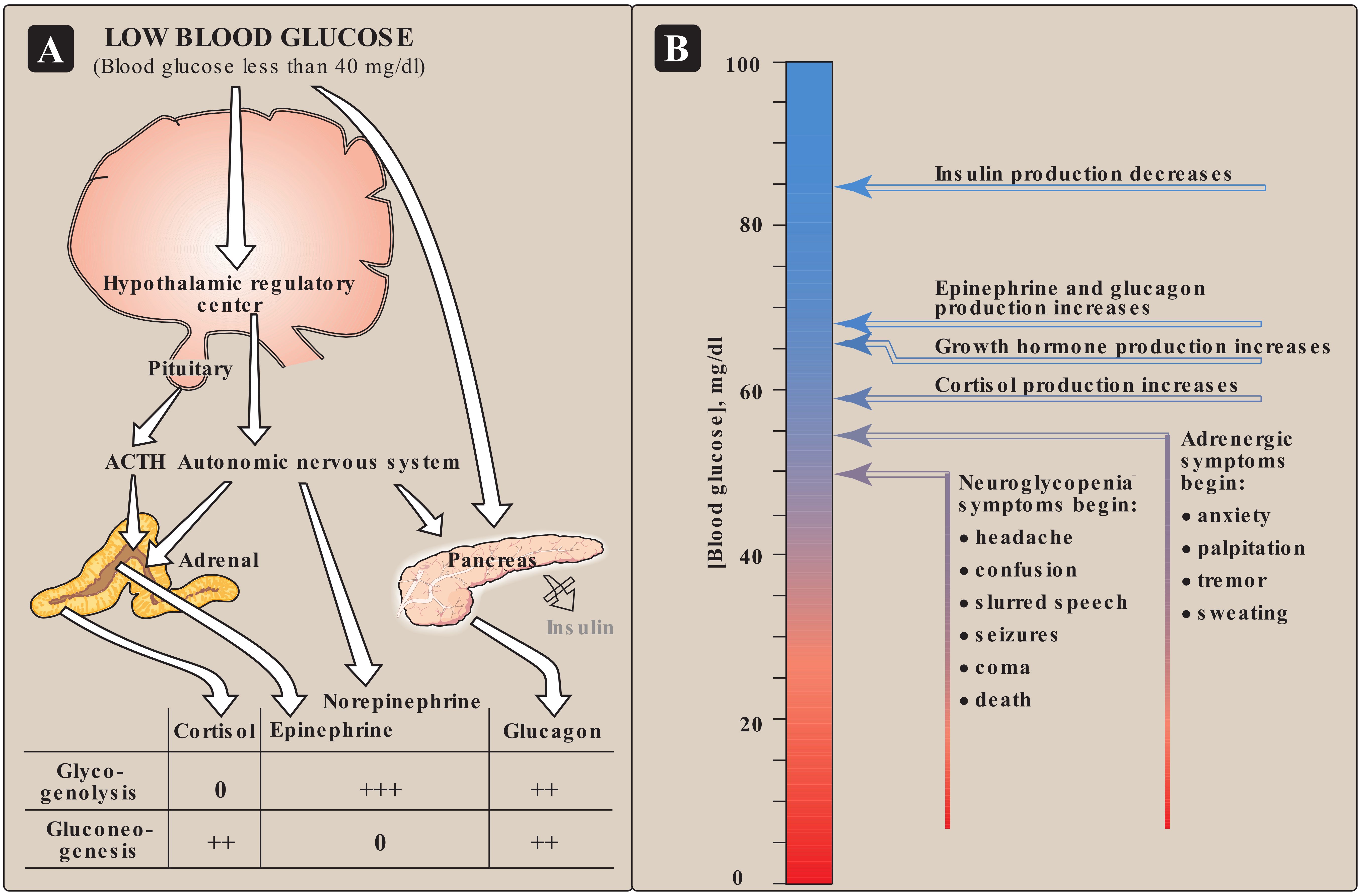
Glucagon binds to high-affinity G protein–coupled receptors on the cell membrane of hepatocytes. The receptors for glucagon are distinct from those that bind insulin or epinephrine. [Note: Glucagon receptors are not found on skeletal muscle.] Glucagon binding results in activation of adenylyl cyclase in the plasma membrane (Figure 23.12; also see p. 94). This causes a rise in cyclic adenosine monophosphate (cAMP), which, in turn, activates cAMP-dependent protein kinase A and increases the phosphorylation of specific enzymes or other proteins. This cascade of increasing enzymic activities results in the phosphorylation-mediated activation or inhibition of key regulatory enzymes involved in carbohydrate and lipid metabolism. An example of such a cascade in glycogen degradation is shown in Figure 11.9 on p. 130. [Note: Glucagon, like insulin, affects gene transcription. For example, glucagon induces expression of phosphoenolpyruvate carboxykinase (see p. 122).]

IV. HYPOGLYCEMIA

Hypoglycemia is characterized by 1) central nervous system (CNS) symptoms, including confusion, aberrant behavior, or coma; 2) a simultaneous blood glucose level equal to or less than 40 mg/dl; and 3) symptoms being resolved within minutes following the administration of glucose (Figure 23.13). Hypoglycemia is a medical emergency because the CNS has an absolute requirement for a continuous supply of bloodborne glucose to serve as fuel for energy metabolism. Transient hypoglycemia can cause cerebral dysfunction, whereas severe, prolonged hypoglycemia causes brain death. Therefore, it is not surprising that the body has multiple overlapping mechanisms to prevent or correct hypoglycemia. The most important hormone changes in combating hypoglycemia are elevated glucagon and the catecholamines, combined with the diminished release of insulin.

A. Symptoms of hypoglycemia

The symptoms of hypoglycemia can be divided into two categories. Adrenergic symptoms, such as anxiety, palpitation, tremor, and sweating, are mediated by catecholamine release (primarily epinephrine) regulated by the hypothalamus in response to hypoglycemia. Adrenergic symptoms typically occur when blood glucose levels


A
LOW BLOOD GLUCOSE
(Blood glucose less than 40 mg/dl)
Hypothalamic regulatory center
Pituitary
ACTH
Autonomic nervous system
Adrenal
Pancreas
Insulin
Norepinephrine
B
100
80
60
40
20
0
[Blood glucose], mg/dl
Insulin production decreases
Epinephrine and glucagon production increases
Growth hormone production increases
Cortisol production increases
Adrenergic symptoms begin:
• anxiety
• palpitation
• tremor
• sweating
Neuroglycopenia symptoms begin:
• headache
• confusion
• slurred speech
• seizures
• coma
• death

	Cortisol	Epinephrine	Glucagon
Glycogenolysis	0	+++	++
Gluconeogenesis	++	0	++

Figure 23.13
A. Actions of some of the glucoregulatory hormones in response to low blood glucose. B. Glycemic thresholds for the various responses to hypoglycemia. [Note: Normal fasted blood glucose is 70–99 mg/100 ml.] + = weak stimulation; ++ = moderate stimulation; +++ = strong stimulation; 0 = no effect; ACTH = adrenocorticotropic hormone.

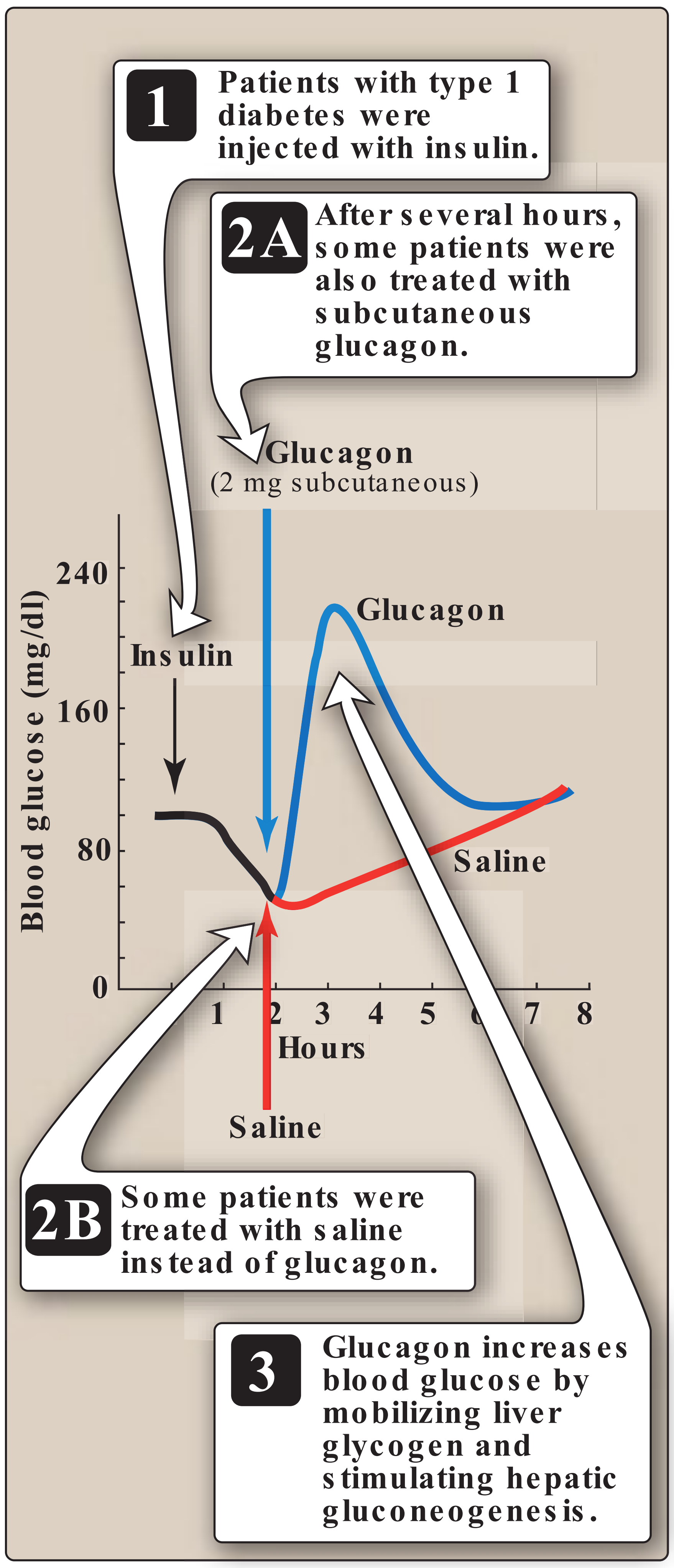
fall abruptly. The second category of hypoglycemic symptoms is neuroglycopenic. Neuroglycopenia (that is, the impaired delivery of glucose to the brain) results in impairment of brain function, causing headache, confusion, slurred speech, seizures, coma, and death. Neuroglycopenic symptoms often result from a gradual decline in blood glucose, often to levels below 40 mg/dl. The slow decline in glucose deprives the CNS of fuel, but fails to trigger an adequate adrenergic response.

B. Glucoregulatory systems

Humans have two overlapping glucose-regulating systems that are activated by hypoglycemia: 1) the pancreatic α cells, which release glucagon, and 2) receptors in the hypothalamus, which respond to abnormally low concentrations of blood glucose. The hypothalamic glucoreceptors can trigger both the secretion of catecholamines (mediated by the autonomic nervous system) and release of adrenocorticotropic hormone (ACTH) and growth hormone by the anterior pituitary (see Figure 23.13). [Note: ACTH increases cortisol synthesis and secretion in the adrenal cortex (see p. 239.] Glucagon, the catecholamines, cortisol, and growth hormones are sometimes called the "counterregulatory" hormones because each opposes the action of insulin on glucose use.

1. **Glucagon and epinephrine:** Secretion of these hormones is most important in the acute, short-term regulation of blood glucose levels. Glucagon stimulates hepatic glycogenolysis and gluconeogenesis. Epinephrine promotes glycogenolysis and lipolysis, inhibits insulin secretion, and inhibits the insulin-mediated uptake of glucose by peripheral tissues. Epinephrine assumes a critical role in hypoglycemia when glucagon secretion is deficient, for example, in the late stages of type 1 diabetes mellitus (see p. 340). The prevention or correction of hypoglycemia fails when the secretion of both glucagon and epinephrine is deficient.

2. **Cortisol and growth hormone:** These hormones are less important in the short-term maintenance of blood glucose concentrations. They do, however, play a role in the long-term (transcriptional) management of glucose metabolism.



1 Patients with type 1 diabetes were injected with insulin.

2A After several hours, some patients were also treated with subcutaneous glucagon.

2B Some patients were treated with saline instead of glucagon.

3 Glucagon increases blood glucose by mobilizing liver glycogen and stimulating hepatic gluconeogenesis.

Figure 23.14
Reversal of insulin-induced hypoglycemia by administration of subcutaneous glucagon.

C. Types of hypoglycemia

Hypoglycemia may be divided into four types: 1) insulin-induced, 2) postprandial (sometimes called reactive hypoglycemia), 3) fasting hypoglycemia, and 4) alcohol-related.

1. **Insulin-induced hypoglycemia:** Hypoglycemia occurs frequently in patients with diabetes who are receiving insulin treatment, particularly those striving to achieve tight control of blood glucose levels. Mild hypoglycemia in fully conscious patients is treated by oral administration of carbohydrate. Unconscious patients are typically given glucagon subcutaneously or intramuscularly (Figure 23.14).

2. **Postprandial hypoglycemia:** This is the second most common form of hypoglycemia. It is caused by an exaggerated insulin release following a meal, prompting transient hypoglycemia with mild adrenergic symptoms. The plasma glucose level returns to normal even if the patient is not fed. The only treatment usually

required is that the patient eats frequent small meals rather than the usual three large meals.

3. **Fasting hypoglycemia:** Low blood glucose during fasting is rare but is more likely to present as a serious medical problem. Fasting hypoglycemia, which tends to produce neuroglycopenic symptoms, may result from a reduction in the rate of glucose production by hepatic glycogenolysis or gluconeogenesis. Thus, low blood glucose levels are often seen in patients with hepatocellular damage or adrenal insufficiency or in fasting individuals who have consumed large quantities of ethanol (see below). Alternately, fasting hypoglycemia may be the result of an increased rate of glucose use by the peripheral tissues due to overproduction of insulin by rare pancreatic tumors. If left untreated, a patient with fasting hypoglycemia may lose consciousness and experience convulsions and coma. [Note: Certain inborn errors of metabolism, for example, defects in fatty acid oxidation, result in fasting hypoglycemia.]

4. **Alcohol-related hypoglycemia:** Alcohol is metabolized in the liver by two oxidation reactions (Figure 23.15). Ethanol is first converted to acetaldehyde by alcohol dehydrogenase. Acetaldehyde is subsequently oxidized to acetate by aldehyde dehydrogenase (ALDH). [Note: ALDH is inhibited by disulfiram, a drug that is used in the treatment of chronic alcoholism. The resulting rise in acetaldehyde results in flushing, tachycardia, hyperventilation, and nausea.] In each reaction, electrons are transferred to oxidized nicotinamide adenine dinucleotide (NAD^+), resulting in an increase in the concentration of cytosolic NADH. The abundance of NADH favors the reduction of pyruvate to lactate and of oxaloacetate (OAA) to malate. Recall from p. 118 that pyruvate and OAA are intermediates in the synthesis of glucose. Thus, the ethanol-mediated increase in NADH causes these intermediates of gluconeogenesis to be diverted into alternate pathways, resulting in the decreased synthesis of glucose. This can precipitate hypoglycemia, particularly in individuals who have depleted their stores of liver gly-

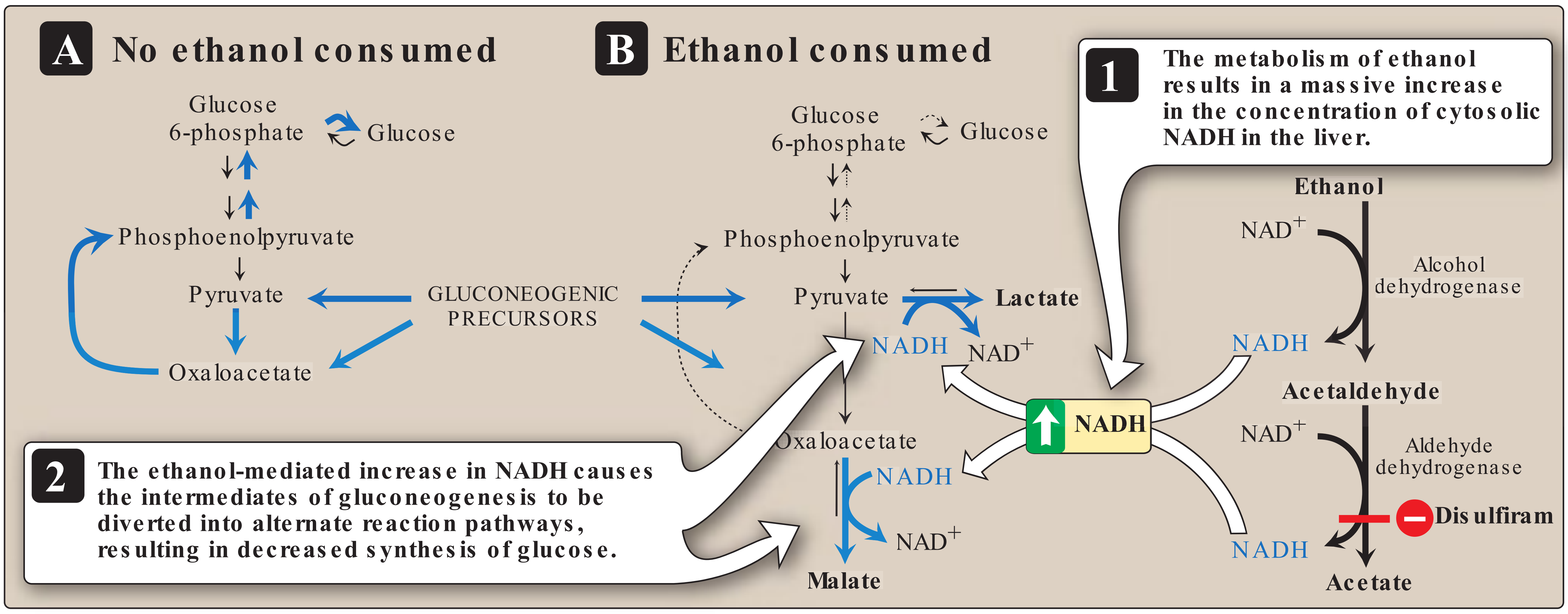

A
No ethanol consumed
Glucose 6-phosphate
Glucose
Phosphoenolpyruvate
Pyruvate
Oxaloacetate
GLUCONEOGENIC PRECURSORS
B
Ethanol consumed
Glucose 6-phosphate
Glucose
Phosphoenolpyruvate
Pyruvate
Lactate
NADH
NAD+
Oxaloacetate
NADH
NAD+
Malate
NADH
1
The metabolism of ethanol results in a massive increase in the concentration of cytosolic NADH in the liver.
Ethanol
NAD+
Alcohol dehydrogenase
NADH
Acetaldehyde
NAD+
Aldehyde dehydrogenase
Disulfiram
NADH
Acetate
2
The ethanol-mediated increase in NADH causes the intermediates of gluconeogenesis to be diverted into alternate reaction pathways, resulting in decreased synthesis of glucose.

Figure 23.15
A. Normal gluconeogenesis in the absence of ethanol consumption. B. Inhibition of gluconeogenesis resulting from hepatic metabolism of ethanol. NAD(H) = nicotinamide adenine dinucleotide.

cogen. [Note: Decreased availability of OAA allows acetyl CoA to be diverted to ketone body synthesis in the liver (see p. 195) and can result in alcoholic ketoacidosis.] Hypoglycemia can produce many of the behaviors associated with alcohol intoxication, such as agitation, impaired judgment, and combativeness. Therefore, alcohol consumption in vulnerable individuals (such as those who are fasted or have engaged in prolonged, strenuous exercise) can produce hypoglycemia that may contribute to the behavioral effects of alcohol. Becuase alcohol consumption can also increase the risk for hypoglycemia in patients using insulin, those in an intensive insulin treatment protocol (see p. 340) are counseled about the increased risk of hypoglycemia that generally occurs many hours after alcohol ingestion. [Note: Chronic alcohol consumption can also result in alcoholic fatty liver due to increased hepatic synthesis of TAGs coupled with impaired formation or release of VLDLs. This occurs as a result of decreased fatty acid oxidation due to a fall in the NAD^+/NADH ratio and increased lipogenesis due to the increased availability of fatty acids (decreased catabolism) and of glyceraldehyde 3-phosphate (the dehydrogenase is inhibited by the low NAD^+/NADH ratio). With continued alcohol consumption, alcoholic fatty liver can progress first to alcoholic hepatitis and then to alcoholic cirrhosis (Figure 23.16).]

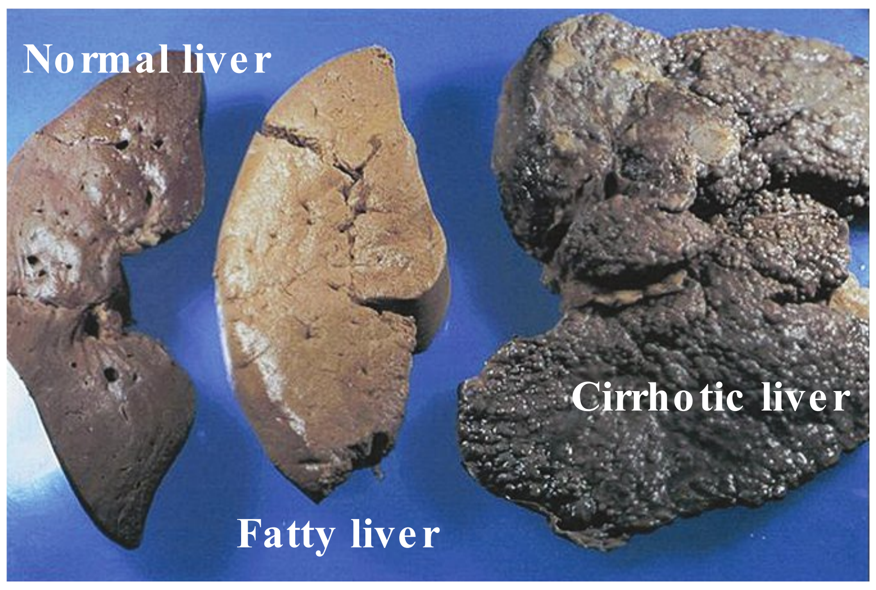


Figure 23.16
Effects of chronic alcohol consumption on liver morphology.

V. CHAPTER SUMMARY

The integration of energy metabolism is controlled primarily by **insulin** and the opposing actions of **glucagon** and the catecholamines, particularly **epinephrine** (Figure 23.17). Changes in the circulating levels of these hormones allow the body to store energy when food is abundant or to make stored energy available in times of physiologic stress (for example, during "survival crises," such as famine). **Insulin** is a peptide hormone produced by the **β cells** of the **islets of Langerhans** of the pancreas. It consists of disulfide-linked A and B chains. A **rise in blood glucose** is the most important signal for insulin **secretion**. The **catecholamines**, secreted in response to stress, trauma, or extreme exercise, **inhibit** insulin **secretion**. Insulin increases glucose uptake (by muscle and adipose) and the synthesis of glycogen, protein, and triacylglycerol: it is an **anabolic** hormone. These actions are mediated by binding to its tyrosine kinase **receptor**. Binding initiates a cascade of cell-signaling responses, including phosphorylation of a family of proteins called **insulin receptor substrate proteins**. **Glucagon** is a monomeric peptide hormone produced by the **α cells** of the pancreatic islets (both insulin and glucagon synthesis involves formation of inactive precursors that are cleaved to form the active hormones). Glucagon, along with epinephrine, norepinephrine, cortisol, and growth hormone (the **"counterregulatory" hormones**), opposes many of the actions of insulin. Glucagon acts to maintain blood glucose during periods of potential hypoglycemia. Glucagon increases glycogenolysis, gluconeogenesis, fatty acid oxidation, ketogenesis, and uptake of amino acids: it is a **catabolic** hormone. Glucagon **secretion** is **stimulated by low blood glucose**, **amino acids**, **and the catecholamines**. Its secretion is **inhibited by elevated blood glucose and by insulin**. Glucagon binds to high-affinity receptors of hepatocytes. Binding results in the activation of adenylyl cyclase, which produces the second messenger **cyclic adenosine monophosphate** (**cAMP**). Subsequent activation of cAMP-dependent protein kinase A results in the phosphorylation-mediated activation or inhibition of key regulatory enzymes involved in carbohydrate and lipid metabolism. Both insulin and glucagon affect **gene transcription**. **Hypoglycemia** is characterized by low blood glucose accompanied by adrenergic and neuroglycopenic symptoms that are rapidly resolved by the administration of glucose. Insulin-induced, postprandial, and fasting hypoglycemia result in release of glucagon and epinephrine. The rise in **nicotinamide adenine dinucleotide** (**NADH**) that accompanies **ethanol** metabolism inhibits gluconeogenesis, leading to hypoglycemia in individuals with depleted stores. Alcohol consumption also increases the risk for hypoglycemia in patients using insulin. Chronic alcohol consumption can cause fatty liver disease.

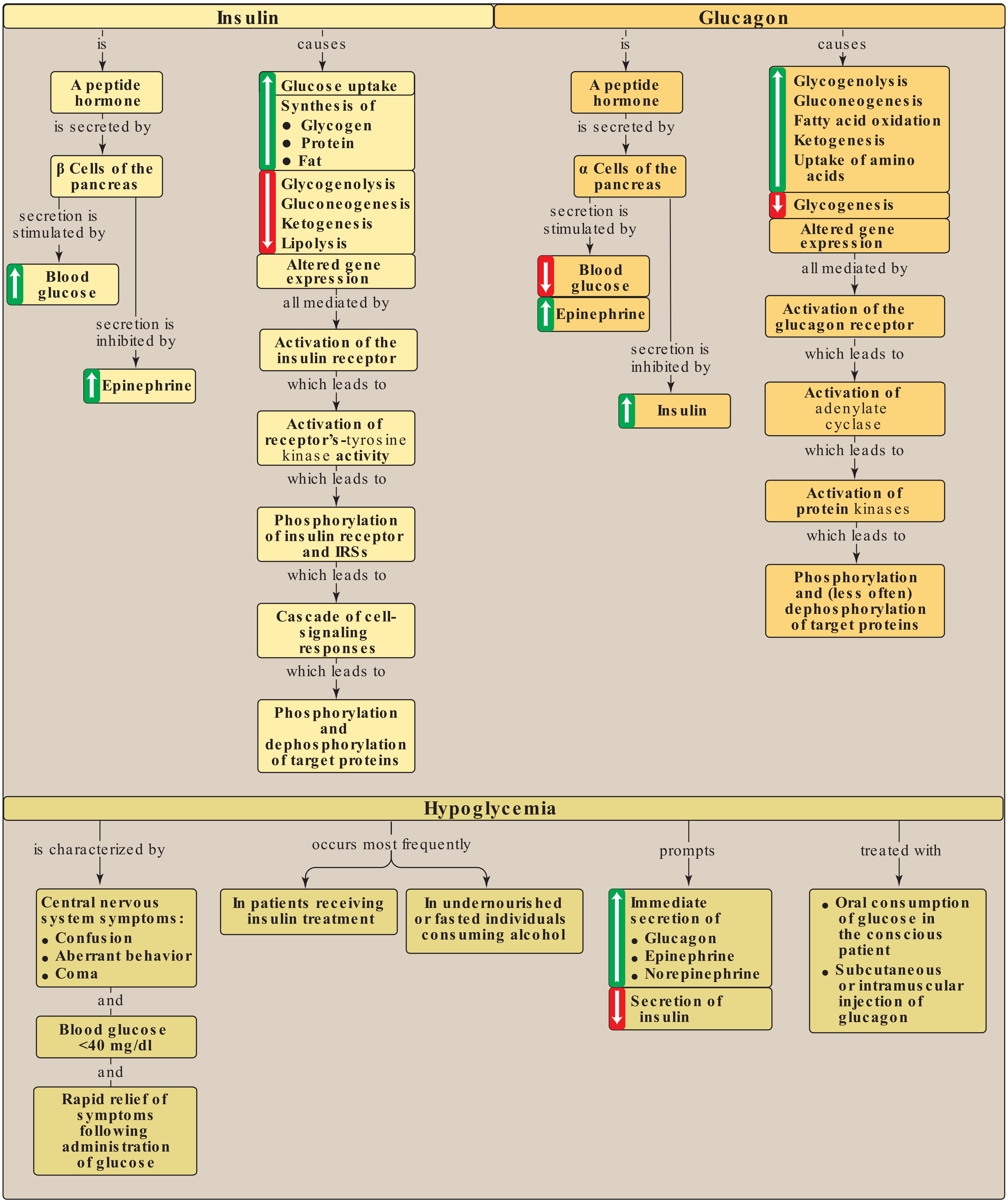

Insulin
is
A peptide hormone
is secreted by
β Cells of the pancreas
secretion is stimulated by
↑ Blood glucose
secretion is inhibited by
↑ Epinephrine
causes
↑ Glucose uptake
Synthesis of
• Glycogen
• Protein
• Fat
↓ Glycogenolysis
Gluconeogenesis
Ketogenesis
Lipolysis
Altered gene expression
all mediated by
Activation of the insulin receptor
which leads to
Activation of receptor's-tyrosine kinase activity
which leads to
Phosphorylation of insulin receptor and IRSs
which leads to
Cascade of cell-signaling responses
which leads to
Phosphorylation and dephosphorylation of target proteins
Glucagon
is
A peptide hormone
is secreted by
α Cells of the pancreas
secretion is stimulated by
↓ Blood glucose
↑ Epinephrine
secretion is inhibited by
↑ Insulin
causes
↑ Glycogenolysis
Gluconeogenesis
Fatty acid oxidation
Ketogenesis
Uptake of amino acids
↓ Glycogenesis
Altered gene expression
all mediated by
Activation of the glucagon receptor
which leads to
Activation of adenylate cyclase
which leads to
Activation of protein kinases
which leads to
Phosphorylation and (less often) dephosphorylation of target proteins
Hypoglycemia
is characterized by
Central nervous system symptoms:
• Confusion
• Aberrant behavior
• Coma
and
Blood glucose <40 mg/dl
and
Rapid relief of symptoms following administration of glucose
occurs most frequently
In patients receiving insulin treatment
In undernourished or fasted individuals consuming alcohol
prompts
↑ Immediate secretion of
• Glucagon
• Epinephrine
• Norepinephrine
↓ Secretion of insulin
treated with
• Oral consumption of glucose in the conscious patient
• Subcutaneous or intramuscular injection of glucagon

Figure 23.17
Key concept map for the metabolic effects of insulin and glucagon, and hypoglycemia. IRSs = insulin receptor substrates.

The Feed–Fast Cycle

24

I. OVERVIEW OF THE ABSORPTIVE STATE

The absorptive (well-fed) state is the 2- to 4-hour period after ingestion of a normal meal. During this interval, transient increases in plasma glucose, amino acids, and triacylglycerols (TAG) occur, the latter primarily as components of chylomicrons synthesized by the intestinal mucosal cells (see p. 228). Islet tissue of the pancreas responds to the elevated levels of glucose with an increased secretion of insulin and a decreased release of glucagon. The elevated insulin-to-glucagon ratio and the ready availability of circulating substrates make the absorptive state an anabolic period characterized by increased synthesis of TAG and glycogen to replenish fuel stores and enhanced synthesis of protein. During this absorptive period, virtually all tissues use glucose as a fuel, and the metabolic response of the body is dominated by alterations in the metabolism of liver, adipose tissue, skeletal muscle, and brain. In this chapter, an "organ map" is introduced that traces the movement of metabolites between tissues. The goal is to create an expanded and clinically useful vision of whole-body metabolism.

II. ENZYMIC CHANGES IN THE ABSORPTIVE STATE

The flow of intermediates through metabolic pathways is controlled by four mechanisms: 1) the availability of substrates; 2) allosteric regulation of enzymes; 3) covalent modification of enzymes; and 4) induction-repression of enzyme synthesis, primarily through regulation of transcription. Although this scheme may at first seem redundant, each mechanism operates on a different timescale (Figure 24.1) and allows the body to adapt to a wide variety of physiologic situations. In the well-fed state, these regulatory mechanisms ensure that available nutrients are captured as glycogen, TAG, and protein.

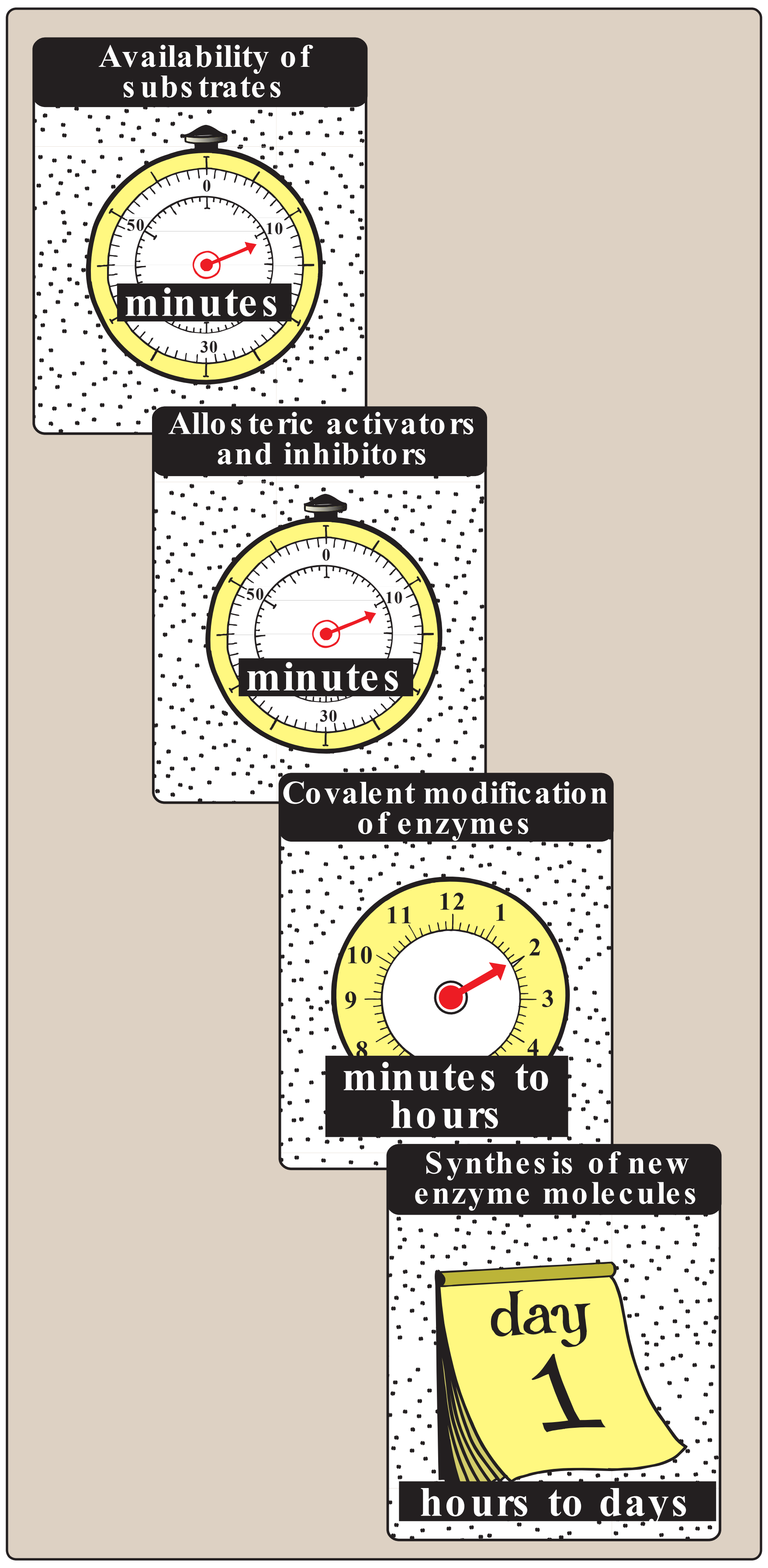

Availability of substrates
minutes
Allosteric activators and inhibitors
minutes
Covalent modification of enzymes
minutes to hours
Synthesis of new enzyme molecules
day 1
hours to days

Figure 24.1
Control mechanisms of metabolism and some typical response times. [Note: Response times may vary according to the nature of the stimulus and from tissue to tissue.]

A. Allosteric effectors

Allosteric changes usually involve rate-determining reactions. For example, glycolysis in the liver is stimulated following a meal by an increase in fructose 2,6-bisphosphate, an allosteric activator of phosphofructokinase-1 ([PFK-1] see p. 99). In contrast, gluconeogenesis is inhibited by fructose 2,6-bisphosphate, an allosteric inhibitor of fructose 1,6-bisphosphatase (see p. 121).

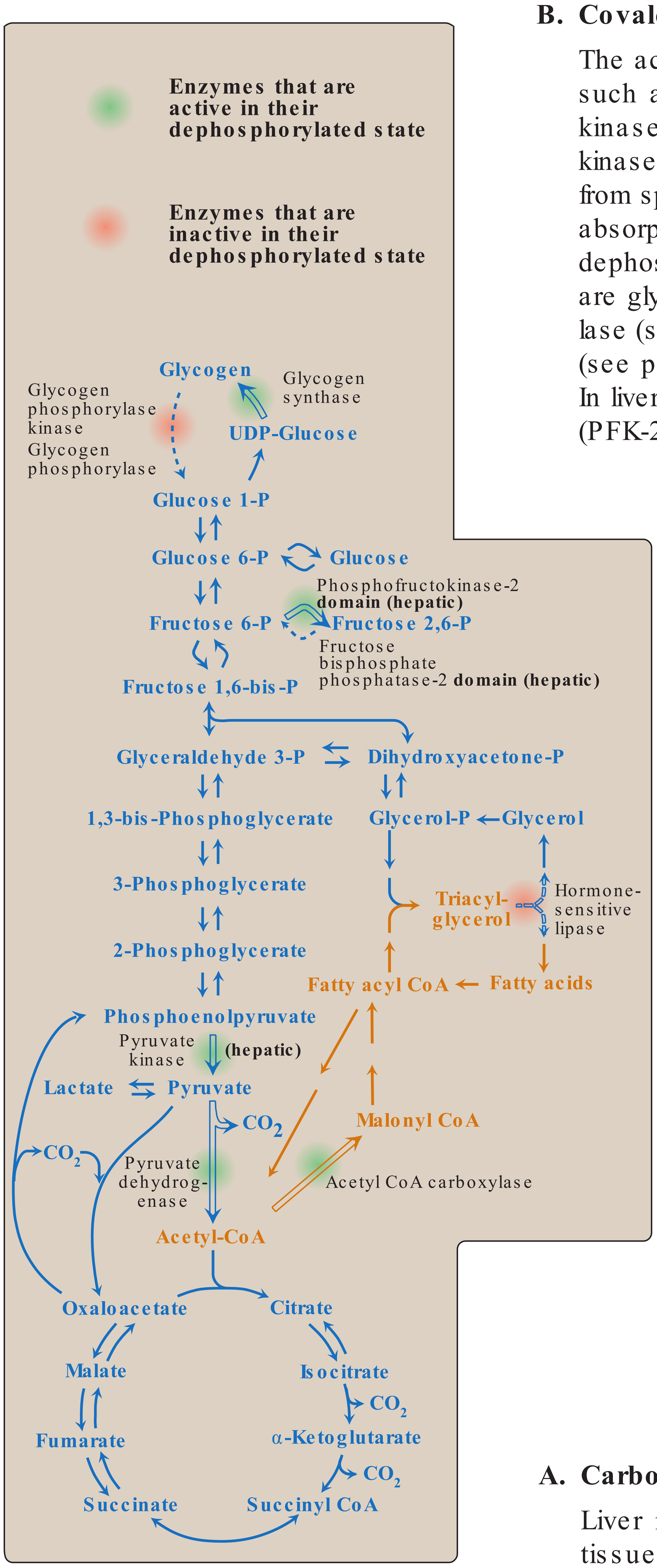

Enzymes that are active in their dephosphorylated state
Enzymes that are inactive in their dephosphorylated state
Glycogen
Glycogen phosphorylase kinase
Glycogen phosphorylase
Glycogen synthase
UDP-Glucose
Glucose 1-P
Glucose 6-P
Glucose
Phosphofructokinase-2 domain (hepatic)
Fructose 6-P
Fructose 2,6-P
Fructose bisphosphate phosphatase-2 domain (hepatic)
Fructose 1,6-bis-P
Glyceraldehyde 3-P
Dihydroxyacetone-P
1,3-bis-Phosphoglycerate
Glycerol-P
Glycerol
3-Phosphoglycerate
Triacyl-glycerol
Hormone-sensitive lipase
2-Phosphoglycerate
Fatty acyl CoA
Fatty acids
Phosphoenolpyruvate
Pyruvate kinase
(hepatic)
Lactate
Pyruvate
CO2
Malonyl CoA
CO2
Pyruvate dehydrogenase
Acetyl CoA carboxylase
Acetyl-CoA
Oxaloacetate
Citrate
Malate
Isocitrate
CO2
Fumarate
α-Ketoglutarate
CO2
Succinate
Succinyl CoA

Figure 24.2

Important reactions of intermediary metabolism regulated by enzyme phosphorylation. **Blue text** = intermediates of carbohydrate metabolism; **brown text** = intermediates of lipid metabolism. P = phosphate; CoA = coenzyme A.

B. Covalent modification

The activity of many enzymes is regulated by the addition (via kinases, such as cyclic adenosine monophosphate [cAMP]-activated protein kinase A [PKA] and adenosine monophosphate-activated protein kinase [AMPK]) or removal (via phosphatases) of phosphate groups from specific serine, threonine, or tyrosine residues of the protein. In the absorptive state, most of the covalently regulated enzymes are in the dephosphorylated form and are active (Figure 24.2). Three exceptions are glycogen phosphorylase kinase (see p. 132), glycogen phosphorylase (see p. 132), and hormone-sensitive lipase (HSL) of adipose tissue (see p. 190), which are inactive in their dephosphorylated form. [Note: In liver, the phosphatase domain of bifunctional phosphofructokinase-2 (PFK-2) is inactive when the protein is dephosphorylated (see p. 100).]

C. Induction and repression of enzyme synthesis

Increased (induction of) or decreased (repression of) enzyme synthesis leads to changes in the number of enzyme molecules, rather than influencing the activity of existing enzyme molecules. Enzymes subject to regulation of synthesis are often those that are needed under specific physiologic conditions. For example, in the fed state, elevated insulin levels result in an increase in the synthesis of key enzymes, such as acetyl coenzyme A (CoA) carboxylase ([ACC] see p. 184) and fatty acid synthase (see p.184), involved in anabolic metabolism. In the fasted state, glucagon induces expression of phosphoenolpyruvate carboxykinase (PEPCK) of gluconeogenesis (see p.120). Both hormones affect transcription factors.

III. LIVER: NUTRIENT DISTRIBUTION CENTER

The liver is uniquely situated to process and distribute dietary nutrients because the venous drainage of the gut and pancreas passes through the hepatic portal vein before entry into the general circulation. Thus, after a meal, the liver is bathed in blood containing absorbed nutrients and elevated levels of insulin secreted by the pancreas. During the absorptive period, the liver takes up carbohydrates, lipids, and most amino acids. These nutrients are then metabolized, stored, or routed to other tissues. In this way, the liver smooths out potentially broad fluctuations in the availability of nutrients for the peripheral tissues.

A. Carbohydrate metabolism

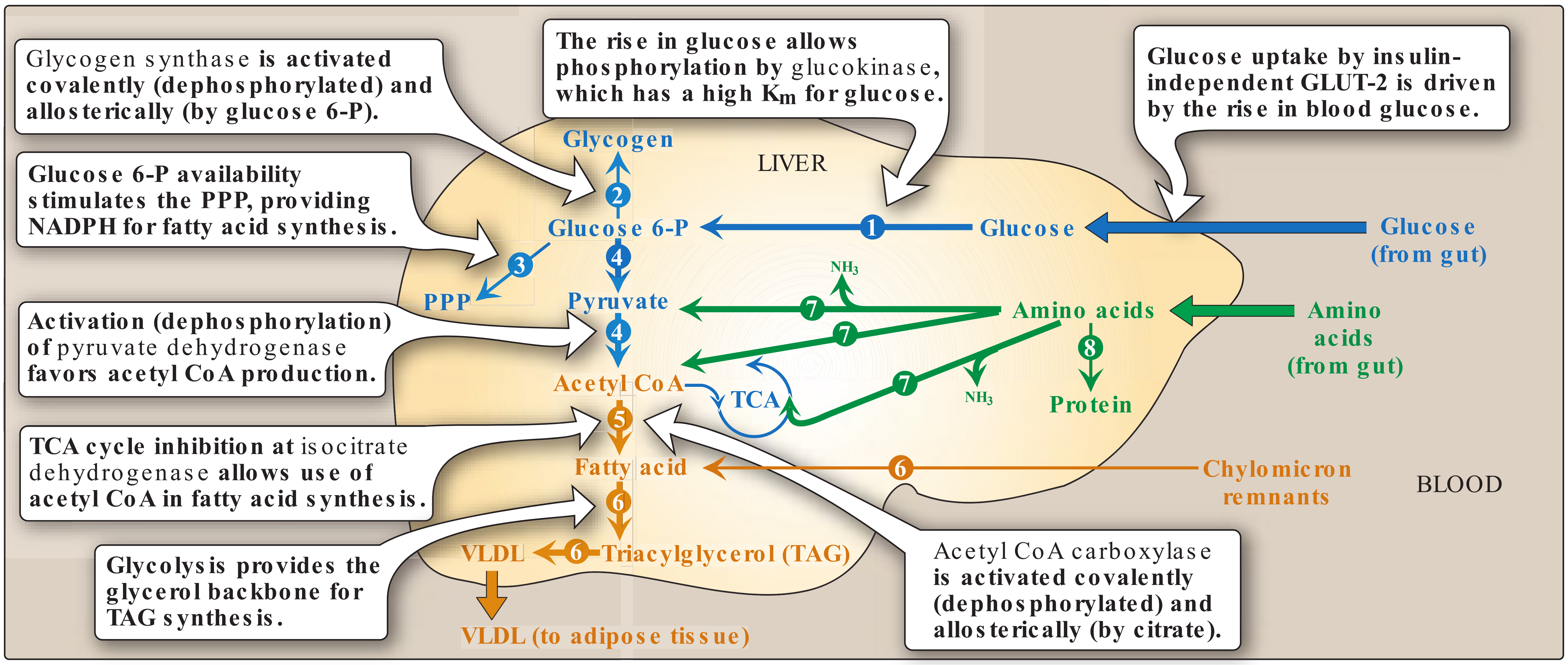
Liver is normally a glucose-producing rather than a glucose-using tissue. However, after a meal containing carbohydrate, the liver becomes a net consumer, retaining roughly 60 of every 100 g of glucose presented by the portal system. This increased use reflects increased glucose uptake by the hepatocytes. Their insulin-independent glucose transporter (GLUT-2) has a low affinity (high K_m) for glucose and, therefore, takes up glucose only when blood glucose is high (see p. 97). Additional mechanisms by which hepatic glucose metabolism is increased include the following. [Note: The numbers in colored circles in the text refer to Figure 24.3.]

1. **Increased phosphorylation of glucose:** The elevated levels of glucose within the hepatocyte (as a result of elevated extracellular levels) allow glucokinase to phosphorylate glucose to glucose 6-phosphate (Figure 24.3, □). (Recall that glucokinase has a high K_m for glucose, is not subject to direct product inhibition, and has a sigmoidal reaction curve; see p.98.).

2. **Increased glycogenesis:** The conversion of glucose 6-phosphate to glycogen is favored by the activation of glycogen synthase, both by dephosphorylation and by increased availability of glucose 6-phosphate, its allosteric effector (see Figure 24.3, □).

3. **Increased activity of the pentose phosphate pathway:** The increased availability of glucose 6-phosphate, combined with the active use of nicotinamide adenine dinucleotide phosphate (NADPH) in hepatic lipogenesis, stimulates the pentose phosphate pathway ([PPP] see p. 145). This pathway typically accounts for 5%–10% of the glucose metabolized by the liver (see Figure 24.3, □).

4. **Increased glycolysis:** In liver, glycolysis is significant only during the absorptive period following a carbohydrate-rich meal. The conversion of glucose to pyruvate is stimulated by the elevated insulin-to-glucagon ratio that results in increased amounts of the regulated enzymes of glycolysis: glucokinase, PFK-1, and pyruvate kinase ([PK] see p. 102). Additionally, PFK-1 is allosterically activated by fructose 2,6-bisphosphate generated by the active (dephosphorylated) kinase domain of bifunctional PFK-2. PK is dephosphorylated and active. Pyruvate dehydrogenase (PDH), which converts pyruvate to acetyl CoA, is active (dephosphorylated) because pyruvate inhibits PDH kinase (see Figure 24.3, □). The acetyl CoA either is



Glycogen synthase is activated covalently (dephosphorylated) and allosterically (by glucose 6-P).

The rise in glucose allows phosphorylation by glucokinase, which has a high K_m for glucose.

Glucose uptake by insulin-independent GLUT-2 is driven by the rise in blood glucose.

Glucose 6-P availability stimulates the PPP, providing NADPH for fatty acid synthesis.

Activation (dephosphorylation) of pyruvate dehydrogenase favors acetyl CoA production.

TCA cycle inhibition at isocitrate dehydrogenase allows use of acetyl CoA in fatty acid synthesis.

Glycolysis provides the glycerol backbone for TAG synthesis.

Acetyl CoA carboxylase is activated covalently (dephosphorylated) and allosterically (by citrate).

Figure 24.3
Major metabolic pathways in liver in the absorptive state. [Note: The acetyl CoA is also used for cholesterol synthesis.] The numbers in circles, which appear both in the figure and in the text, indicate important pathways for carbohydrate, fat, or protein metabolism. **Blue text** = intermediates of carbohydrate metabolism; **brown text** = intermediates of lipid metabolism; **green text** = intermediates of protein metabolism. P = phosphate; PPP = pentose phosphate pathway; TCA = tricarboxylic acid (cycle); CoA = coenzyme A; VLDL = very-low-density lipoprotein; GLUT = glucose transporter; NADPH = nicotinamide adenine dinucleotide phosphate.

used as a substrate for fatty acid (FA) synthesis or is oxidized for energy in the tricarboxylic acid (TCA) cycle. (See Figure 24.4 for the central role of glucose 6-phosphate.)

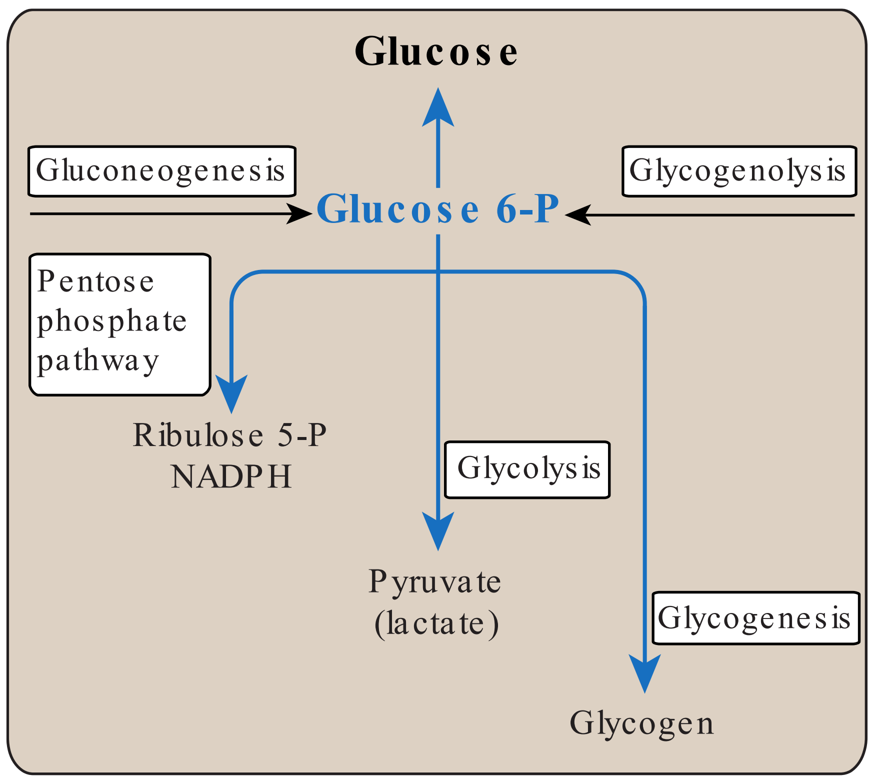


Figure 24.4
Central role of glucose 6-phosphate in metabolism. [Note: The presence of glucose 6-phosphatase in liver allows the production of free glucose from glycogenolysis and gluconeogenesis.] NADPH = nicotinamide adenine dinucleotide phosphate; P = phosphate.

5. **Decreased production of glucose:** Although glycolysis and glycogenesis (pathways that promote glucose storage) are stimulated in liver in the absorptive state, gluconeogenesis and glycogenolysis (pathways that generate glucose) are decreased. Pyruvate carboxylase (PC), which catalyzes the first step in gluconeogenesis, is largely inactive due to low levels of acetyl CoA, its allosteric activator (see p. 119). [Note: The acetyl CoA is being used for fatty acid synthesis.] The high insulin-to-glucagon ratio also favors inactivation of other gluconeogenic enzymes such as fructose 1,6-bisphosphatase (see Figure 8.17, p. 100). Glycogenolysis is inhibited by dephosphorylation of glycogen phosphorylase and phosphorylase kinase.

B. Fat metabolism

1. **Increased fatty acid synthesis:** Liver is the primary tissue for de novo synthesis of FAs (see Figure 24.3, □). FA synthesis, a cytosolic process, is favored in the absorptive period by availability of the substrates acetyl CoA (from glucose and amino acid metabolism) and NADPH (from glucose metabolism) and by the activation of ACC, both by dephosphorylation and by the presence of its allosteric activator, citrate. [Note: Inactivity of AMPK favors dephosphorylation.] ACC catalyzes the formation of malonyl CoA from acetyl CoA, the rate-limiting reaction for FA synthesis (see p. 183). [Note: Malonyl CoA inhibits carnitine palmitoyltransferase-I (CPT-I) of FA oxidation (see p.191). Citrate, thereby, directly activates FA synthesis and indirectly inhibits FA degradation.]

   a. **Source of cytosolic acetyl coenzyme A:** Pyruvate from aerobic glycolysis enters mitochondria and is decarboxylated by PDH. The acetyl CoA product is combined with oxaloacetate (OAA) to form citrate via citrate synthase. Citrate leaves the mitochondria (as a result of the inhibition of isocitrate dehydrogenase by adenosine triphosphate [ATP]) and enters the cytosol. Citrate is cleaved by ATP-citrate lyase (induced by insulin), producing the acetyl CoA substrate of ACC and OAA. The OAA is reduced to malate, which is oxidatively decarboxylated to pyruvate by malic enzyme as NADPH is formed (see p. 187).

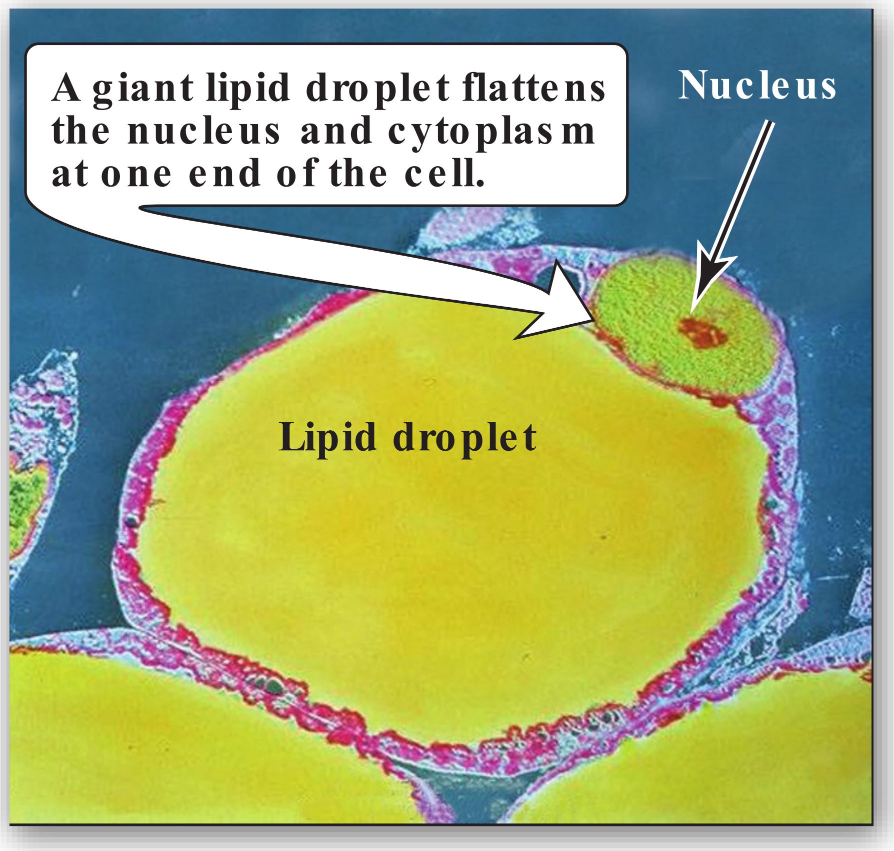

A giant lipid droplet flattens the nucleus and cytoplasm at one end of the cell.
Nucleus
Lipid droplet

Figure 24.5
Colorized transmission electron micrograph of adipocytes.

2. **Increased triacylglycerol synthesis:** TAG synthesis is favored because fatty acyl CoAs are available both from de novo synthesis from acetyl CoA and from hydrolysis of the TAG component of chylomicron remnants removed from the blood by hepatocytes (see p. 178). Glycerol 3-phosphate, the backbone for TAG synthesis, is provided by glycolysis (see p. 189). The liver packages TAG into very-low-density lipoprotein (VLDL) particles that are secreted into the blood for use by extrahepatic tissues, particularly adipose and muscle tissues (see Figure 24.3, □).

C. Amino acid metabolism

1. **Increased amino acid degradation:** In the absorptive period, more amino acids are present than the liver can use in the synthesis

of proteins and other nitrogen-containing molecules. The surplus amino acids are not stored but are either released into the blood for other tissues to use in protein synthesis or deaminated, with the resulting carbon skeletons being degraded by the liver to pyruvate, acetyl CoA, or TCA cycle intermediates. These metabolites can be oxidized for energy or used in FA synthesis (see Figure 24.3, □). The liver has limited capacity to degrade the branched-chain amino acids (BCAAs) leucine, isoleucine, and valine. They pass through the liver essentially unchanged and are preferentially metabolized in muscle (see p. 266).

2. **Increased protein synthesis:** The body does not store protein in the same way that it maintains glycogen or TAG reserves (see p. 327). However, a transient increase in the synthesis of hepatic proteins does occur in the absorptive state, resulting in replacement of any proteins that may have been degraded during the previous postabsorptive period (see Figure 24.3, □).

IV. ADIPOSE TISSUE: ENERGY STORAGE DEPOT

Adipose tissue is second only to the liver in its ability to distribute fuel molecules. In a 70-kg man, white adipose tissue (WAT) weighs approximately 14 kg, or about half as much as the total muscle mass. Nearly the entire volume of each adipocyte in WAT can be occupied by a droplet of TAG (Figure 24.5).

A. Carbohydrate metabolism

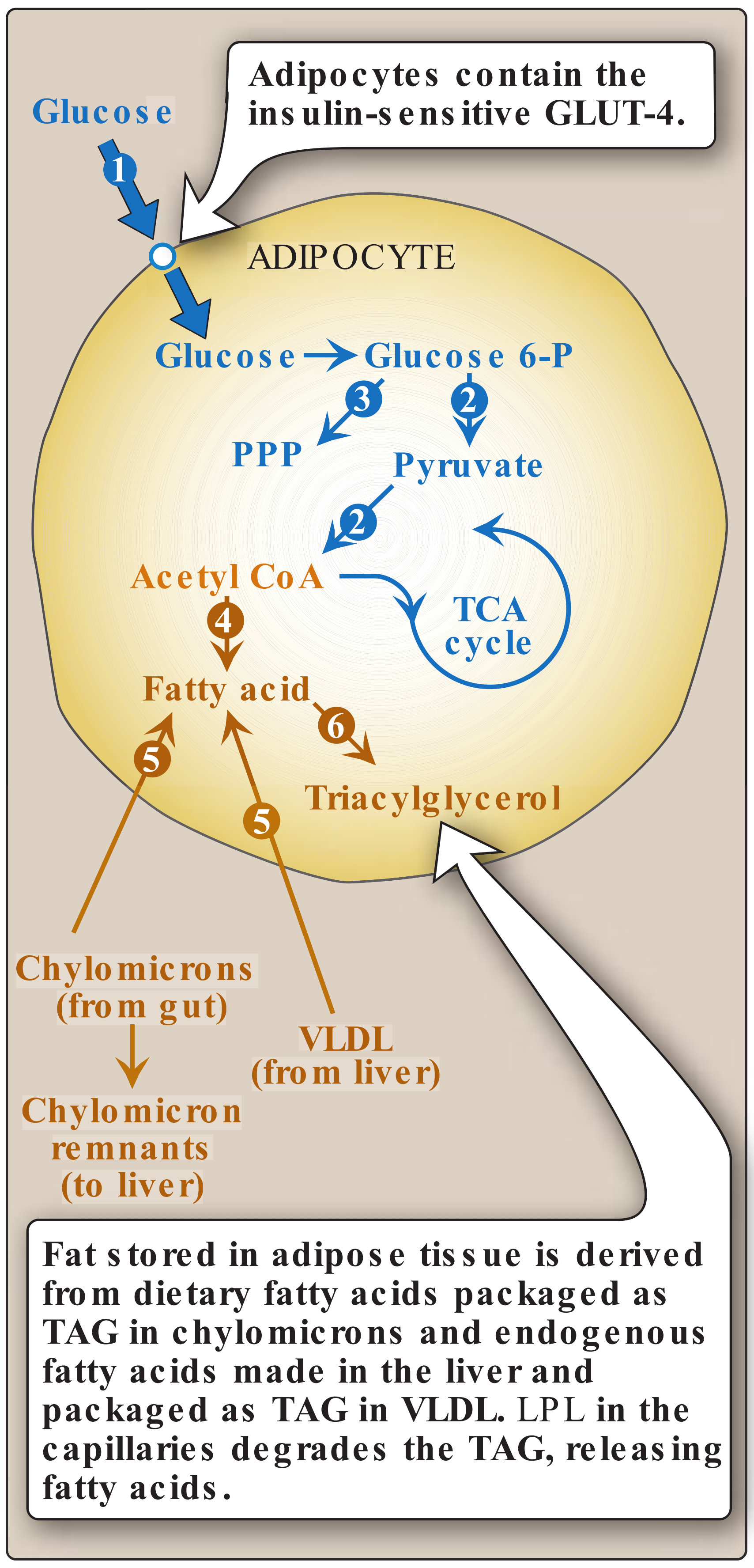
1. **Increased glucose transport:** Circulating insulin levels are elevated in the absorptive state, resulting in an influx of glucose into adipocytes via insulin-sensitive GLUT-4 recruited to the cell surface from intracellular vesicles (Figure 24.6, □). The glucose is phosphorylated by hexokinase.

2. **Increased glycolysis:** The increased intracellular availability of glucose results in an enhanced rate of glycolysis (see Figure 24.6, □). In adipose tissue, glycolysis serves a synthetic function by supplying glycerol 3-phosphate for TAG synthesis (see p. 189). Recall that adipose tissue lacks glycerol kinase.

3. **Increased activity of the pentose phosphate pathway:** Adipose tissue can metabolize glucose by means of the PPP, thereby producing NADPH, which is essential for fat synthesis (see p. 186 and Figure 24.6, □). However, in humans, de novo synthesis is not a major source of FA in adipose tissue, except when refeeding a previously fasted individual (see Figure 24.6, □).

B. Fat metabolism

Most of the FAs added to the TAG stores of adipocytes after consumption of a lipid-containing meal are provided by the degradation of exogenous (dietary) TAG in chylomicrons sent out by the intestine and endogenous TAG in VLDL sent out by the liver (see Figure 24.6, □). The FAs are released from the lipoproteins by the action of lipoprotein lipase (LPL), an extracellular enzyme attached to the capillary walls in many tissues, particularly adipose and muscle. In adipose



Adipocytes contain the insulin-sensitive GLUT-4.

Fat stored in adipose tissue is derived from dietary fatty acids packaged as TAG in chylomicrons and endogenous fatty acids made in the liver and packaged as TAG in VLDL. LPL **in the capillaries degrades the TAG, releasing fatty acids.**

Figure 24.6
Major metabolic pathways in adipose tissue in the absorptive state. [Note: The numbers in the circles, which appear both in the figure and in the corresponding text, indicate important pathways for adipose tissue metabolism.] GLUT = glucose transporter; P = phosphate; PPP = pentose phosphate pathway; CoA = coenzyme A; TCA = tricarboxylic acid; TAG = triacylglycerol; VLDL = very-low-density lipoprotein; LPL = lipoprotein lipase.

tissue, LPL is upregulated by insulin. Thus, in the fed state, elevated levels of glucose and insulin favor storage of TAG (see Figure 24.6, □), all the carbons of which are supplied by glucose. [Note: Elevated insulin favors the dephosphorylated (inactive) form of HSL (see p. 190), thereby inhibiting lipolysis in the fed state.]

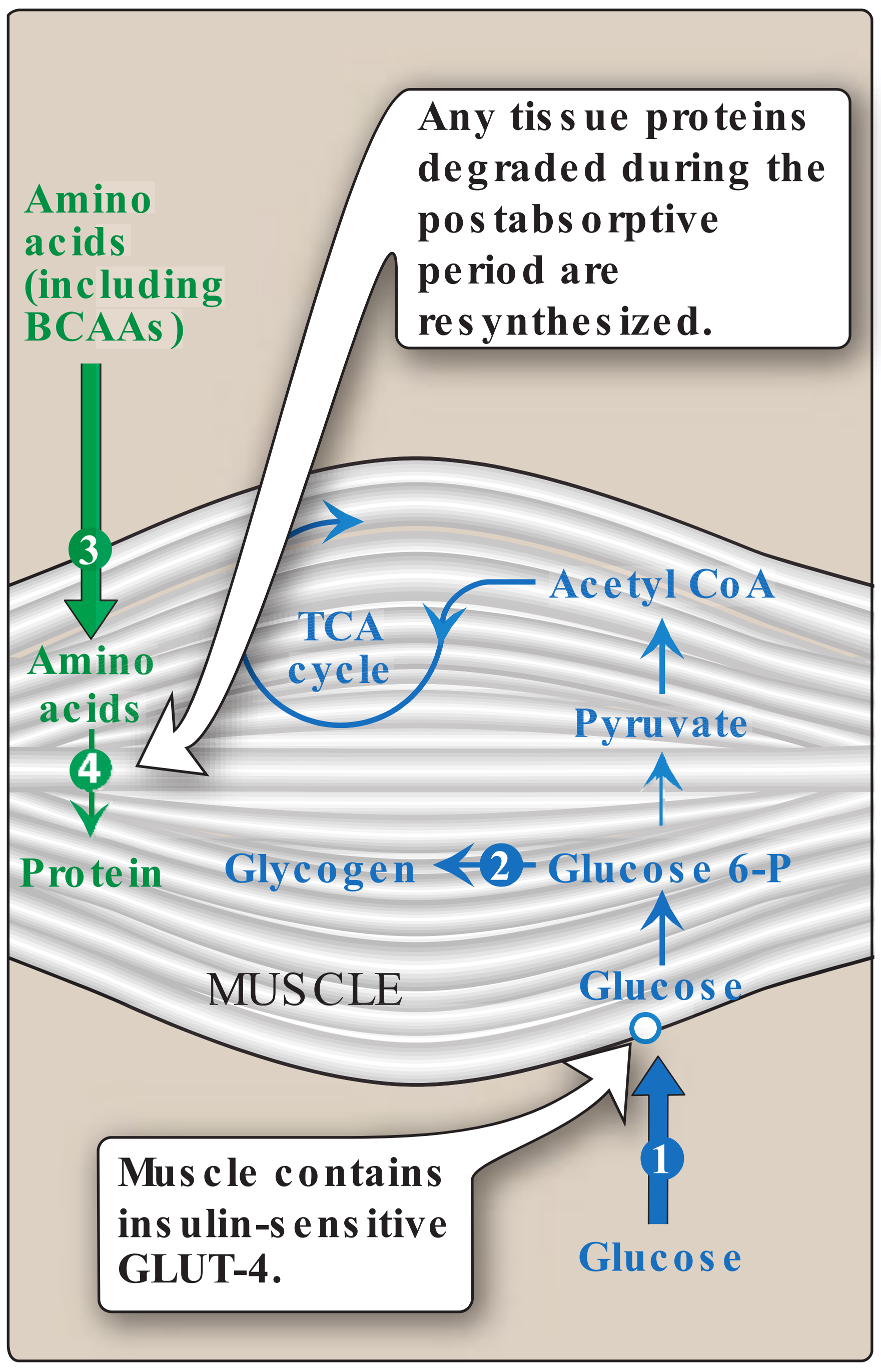

Amino acids (including BCAAs)
Any tissue proteins degraded during the postabsorptive period are resynthesized.
3
Amino acids
4
Protein
TCA cycle
Acetyl CoA
Pyruvate
Glycogen
2
Glucose 6-P
MUSCLE
Glucose
1
Glucose
Muscle contains insulin-sensitive GLUT-4.

Figure 24.7
Major metabolic pathways in skeletal muscle in the absorptive state. [Note: The numbers in circles, which appear both in the figure and in the text, indicate important pathways for carbohydrate or protein metabolism.] CoA = coenzyme A; P = phosphate; GLUT = glucose transporter; BCAAs = branched-chain amino acids; TCA = tricarboxylic acid.

V. RESTING SKELETAL MUSCLE

In the fed state, muscle takes up glucose via GLUT-4 (for energy and glycogen synthesis) and amino acids (for energy and protein synthesis). [Note: The energy metabolism of skeletal muscle is unique in being able to respond to substantial changes in the demand for ATP that accompanies muscle contraction. At rest, muscle accounts for approximately 30% of the O_2 consumption of the body, whereas during vigorous exercise, it is responsible for up to 90% of the total O_2 consumption. This graphically illustrates the fact that skeletal muscle, despite its potential for transient periods of anaerobic glycolysis, is an oxidative tissue. In contrast to liver, there is no covalent regulation of PFK-2 in skeletal muscle. In the cardiac isozyme, however, the kinase domain is activated by epinephrine-mediated phosphorylation.]

A. Carbohydrate metabolism

1. **Increased glucose transport:** The transient increase in plasma glucose and insulin after a carbohydrate-rich meal leads to an increase in glucose transport into muscle cells by GLUT-4 (see p. 97 and Figure 24.7, □), thereby reducing blood glucose. Glucose is phosphorylated to glucose 6-phosphate by hexokinase and metabolized to provide the energy needs of the cells.

2. **Increased glycogen synthesis:** The increased insulin-to-glucagon ratio and the availability of glucose 6-phosphate favor glycogen synthesis, particularly if glycogen stores have been depleted as a result of exercise (see p. 126, and Figure 24.7, □).

B. Fat metabolism

FAs are released from chylomicrons and VLDL by the action of LPL (see pp. 228 and 231). However, fatty acids are of secondary importance as a fuel for resting muscle during the fed state, in which glucose is the primary source of energy.

C. Amino acid metabolism

1. **Increased protein synthesis:** An increase in amino acid uptake and protein synthesis occurs in the absorptive period after ingestion of a meal containing protein (see Figure 24.7, □ and □). This synthesis replaces protein degraded since the previous meal.

2. **Increased uptake of branched-chain amino acids:** Muscle is the principal site for degradation of the BCAAs (leucine, isoleucine, and valine) because it contains the required transaminase (see p. 266). The BCAAs escape metabolism by the liver and are taken up by muscle, where they are used for protein synthesis (see Figure 24.7, □) and as sources of energy.

VI. BRAIN

Although contributing only 2% of the adult weight, the brain accounts for a consistent 20% of the basal O_2 consumption of the body at rest. Because the brain is vital to the proper functioning of all organs of the body, special priority is given to its fuel needs. To provide energy, substrates must be able to cross the endothelial cells that line the blood vessels in the brain (the blood–brain barrier [BBB]). In the fed state, the brain exclusively uses glucose as a fuel (GLUT-1 of the BBB is insulin independent), completely oxidizing approximately 140 g/day to CO_2 and H_2O. The brain contains no significant stores of glycogen and is, therefore, completely dependent on the availability of blood glucose (Figure 24.8, □). [Note: If blood glucose levels fall below 40 mg/100 ml (normal fasted blood glucose is 70–99 mg/100 ml), cerebral function is impaired (see p. 315).] The brain also lacks significant stores of TAG, and the FAs circulating in the blood make little contribution to energy production because FAs bound to albumin do not efficiently cross the BBB. The intertissue exchanges characteristic of the absorptive period are summarized in Figure 24.9.

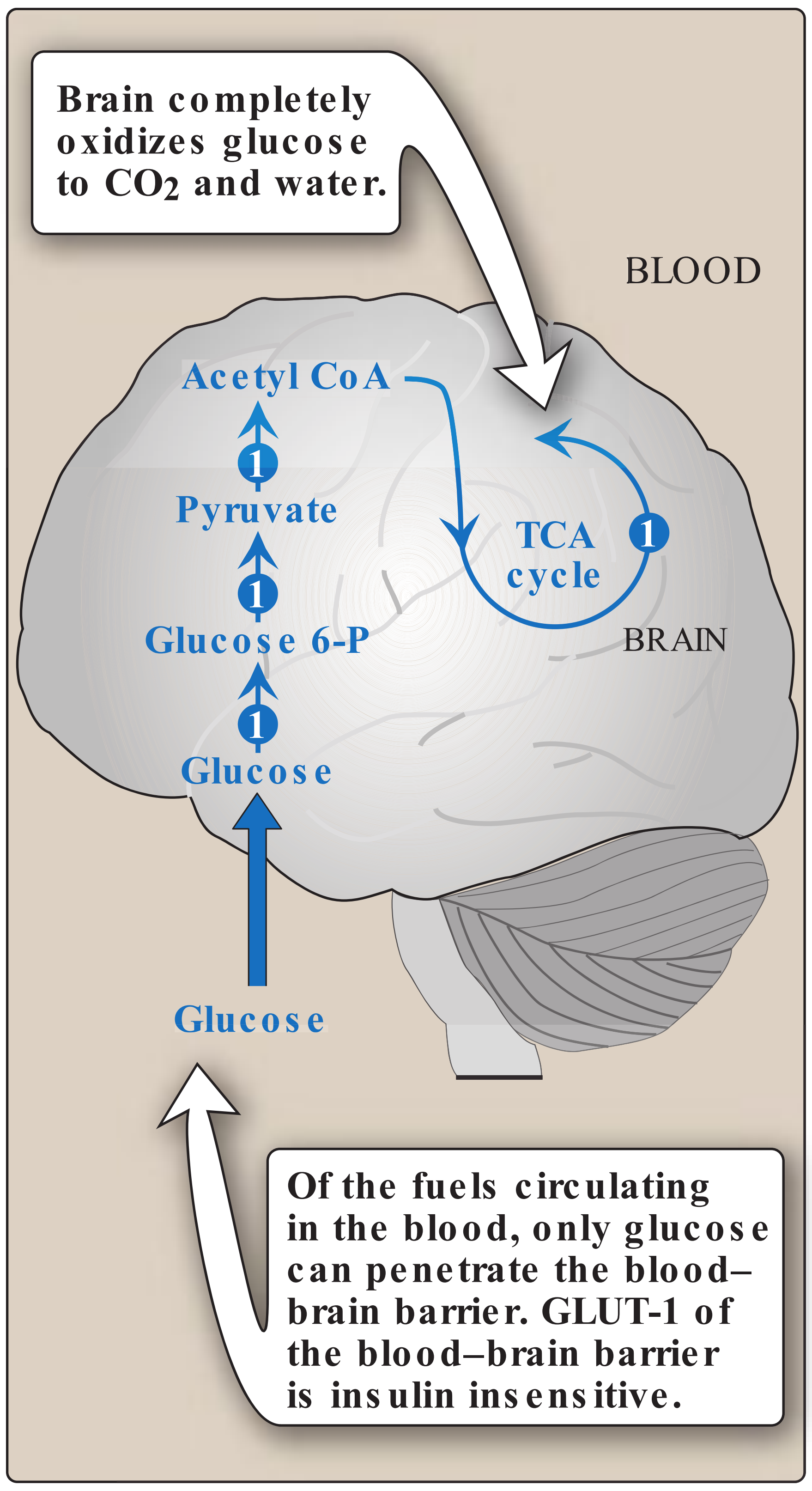

Brain completely oxidizes glucose to CO_2 and water.
BLOOD
Acetyl CoA
①
Pyruvate
①
Glucose 6-P
①
Glucose
TCA cycle
①
BRAIN
Glucose
Of the fuels circulating in the blood, only glucose can penetrate the blood–brain barrier. GLUT-1 of the blood–brain barrier is insulin insensitive.

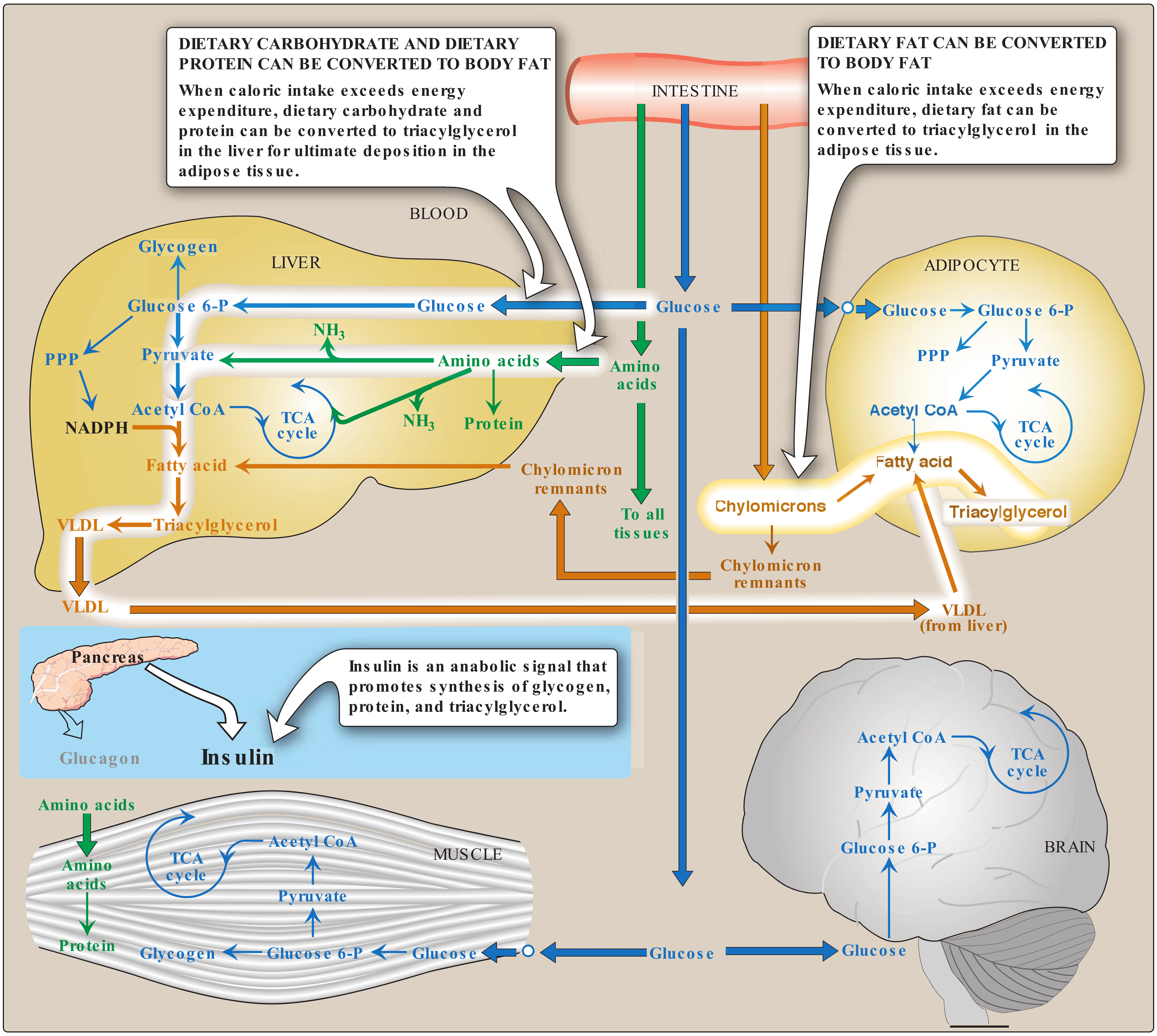
Figure 24.8
Major metabolic pathways in brain in the absorptive state. [Note: The numbers in circles, which appear both in the figure and in the text, indicate important pathways for carbohydrate metabolism.] CoA = coenzyme A; TCA = tricarboxylic acid; P = phosphate; GLUT = glucose transporter.

VII. OVERVIEW OF FASTING

Fasting begins if no food is ingested after the absorptive period. It may result from an inability to obtain food, the desire to lose weight rapidly, or clinical situations in which an individual cannot eat (for example, because of trauma, surgery, cancer, or burns). In the absence of food, plasma levels of glucose, amino acids, and TAG fall, triggering a decline in insulin secretion and an increase in glucagon and epinephrine release. The decreased insulin/counterregulatory hormone ratio and the decreased availability of circulating substrates make the period of nutrient deprivation a catabolic period characterized by degradation of TAG, glycogen, and protein. This sets into motion an exchange of substrates among liver, adipose tissue, skeletal muscle, and brain that is guided by two priorities: 1) the need to maintain adequate plasma levels of glucose to sustain energy metabolism in the brain, red blood cells, and other glucose-requiring tissues and 2) the need to mobilize fatty acids from adipose tissue and the synthesis and release of ketone bodies from the liver to supply energy to other tissues. [Note: Maintaining glucose requires that the substrates for gluconeogenesis (such as pyruvate, alanine, and glycerol) be available.]

A. Fuel stores

The metabolic fuels available in a normal 70-kg man at the beginning of a fast are shown in Figure 24.10. Note the enormous caloric stores available in the form of TAG compared with those contained in glycogen. [Note: Although protein is listed as an energy source, each protein also has a function (for example, as a structural component of the body, an enzyme, and so forth). Therefore, only about one third of the body's protein can be used for energy production without fatally compromising vital functions.]



DIETARY CARBOHYDRATE AND DIETARY PROTEIN CAN BE CONVERTED TO BODY FAT

When caloric intake exceeds energy expenditure, dietary carbohydrate and protein can be converted to triacylglycerol in the liver for ultimate deposition in the adipose tissue.

DIETARY FAT CAN BE CONVERTED TO BODY FAT

When caloric intake exceeds energy expenditure, dietary fat can be converted to triacylglycerol in the adipose tissue.

Insulin is an anabolic signal that promotes synthesis of glycogen, protein, and triacylglycerol.

INTESTINE
BLOOD
LIVER
Glycogen
Glucose 6-P
Glucose
PPP
NADPH
Pyruvate
NH_3
Amino acids
Acetyl CoA
TCA cycle
NH_3
Protein
Fatty acid
Chylomicron remnants
VLDL
Triacylglycerol
VLDL
Glucose
Amino acids
To all tissues
ADIPOCYTE
Glucose
Glucose 6-P
PPP
Pyruvate
Acetyl CoA
TCA cycle
Fatty acid
Chylomicrons
Triacylglycerol
Chylomicron remnants
VLDL (from liver)
Pancreas
Glucagon
Insulin
Amino acids
Amino acids
Protein
TCA cycle
Acetyl CoA
Pyruvate
MUSCLE
Glycogen
Glucose 6-P
Glucose
Glucose
Glucose
BRAIN
Acetyl CoA
TCA cycle
Pyruvate
Glucose 6-P

Figure 24.9
Intertissue relationships in the absorptive state and the hormonal signals that promote them. [Note: Small circles on the perimeter of muscle and the adipocyte indicate insulin-dependent glucose transporters.] P = phosphate; PPP = pentose phosphate pathway; CoA = coenzyme A; NADPH = nicotinamide adenine dinucleotide phosphate; TCA = tricarboxylic acid; VLDL = very-low-density lipoprotein.

B. Enzymic changes in fasting

In fasting (as in the fed state), the flow of intermediates through the pathways of energy metabolism is controlled by four mechanisms: 1) the availability of substrates, 2) allosteric regulation of enzymes, 3) covalent modification of enzymes, and 4) induction–repression of enzyme synthesis. The metabolic changes observed in fasting are generally opposite to those described for the absorptive state (see Figure 24.9). For example, although most of the enzymes regulated by covalent modification are dephosphorylated and active in the fed state, they are phosphorylated and inactive in the fasted state. Three exceptions are glycogen phosphorylase (see p. 132), glycogen phosphorylase kinase (see p. 132), and HSL of adipose tissue (see p. 190), which are active in their phosphorylated states. In fasting, substrates are not provided by the diet but are available from the breakdown of stores and/or tissues, such as glycogenolysis with release of glucose from liver, lipolysis with release of FAs and glycerol from TAG in adipose tissue, and proteolysis with release of amino acids from muscle. Recognition that the changes in fasting are the reciprocal of those in the fed state is helpful in understanding the ebb and flow of metabolism.

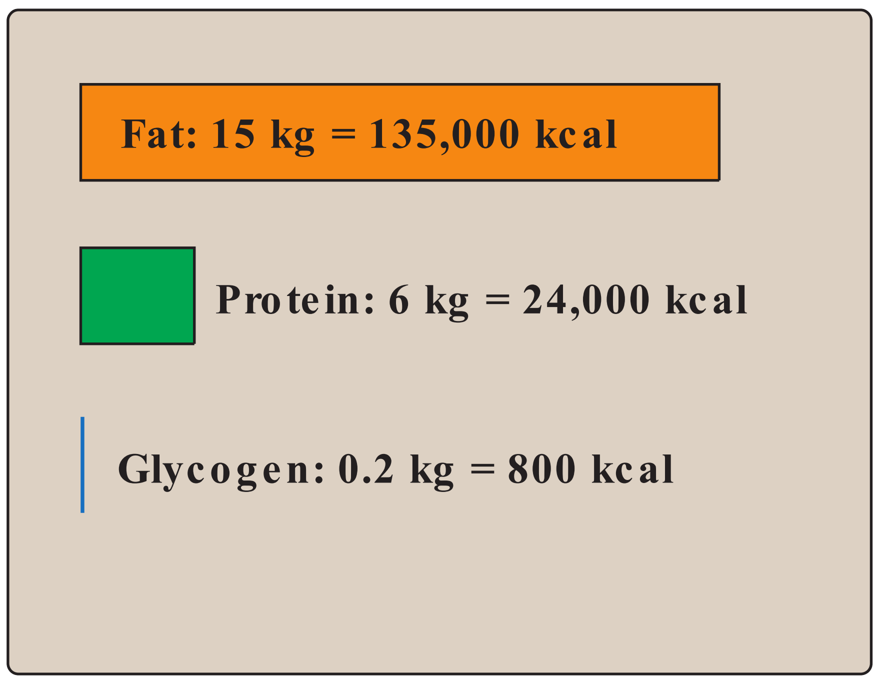


Figure 24.10
Metabolic fuels present in a 70-kg man at the beginning of a fast. The fat stores are sufficient to meet energy needs for about 80 days.

VIII. LIVER IN FASTING

The primary role of liver in energy metabolism during fasting is maintenance of blood glucose through the production of glucose from glycogenolysis and gluconeogenesis for glucose-dependent tissues and the synthesis and distribution of ketone bodies for use by other tissues. Therefore, "hepatic" metabolism and "extrahepatic" or "peripheral" metabolism are distinguished.

A. Carbohydrate metabolism

The liver first uses glycogen degradation and then gluconeogenesis to maintain blood glucose levels to sustain energy metabolism of the brain and other glucose-requiring tissues in the fasted (postabsorptive) state. [Note: Recall that the presence of glucose 6-phosphatase in the liver allows the production of free glucose both from glycogenolysis and from gluconeogenesis (see Figure 24.4).]

1. **Increased glycogen degradation:** Figure 24.11 shows the sources of blood glucose after ingestion of 100 g of glucose. During the brief absorptive period, ingested glucose is the major source of blood glucose. Several hours later, blood glucose levels have declined sufficiently to cause increased secretion of glucagon and decreased release of insulin. The increased glucagon-to-insulin ratio causes a rapid mobilization of liver glycogen stores (which contain about 80 g of glycogen in the fed state) due to PKA-mediated phosphorylation (and activation) of glycogen phosphorylase kinase that phosphorylates (and activates) glycogen phosphorylase (see p. 130). Figure 24.11 shows that liver glycogen is nearly exhausted after 10–18 hours of fasting, and therefore, hepatic glycogenolysis is a transient response to early fasting. Figure 24.12, □, shows glycogen degradation as part of the overall metabolic response of the liver during fasting. [Note: Phosphorylation of glycogen synthase simultaneously inhibits glycogenesis.]

2. **Increased glucose synthesis:** The synthesis of glucose and its release into the circulation are vital hepatic functions during short- and long-term fasting (see Figure 24.12, □). The carbon skeletons for gluconeogenesis are derived primarily from glucogenic amino acids and lactate from muscle and glycerol from adipose tissue. Gluconeogenesis, favored by activation of fructose 1,6-bisphosphatase (due to decreased availability of its inhibitor fructose 2,6-bisphosphate; see p. 121) and by induction of PEPCK

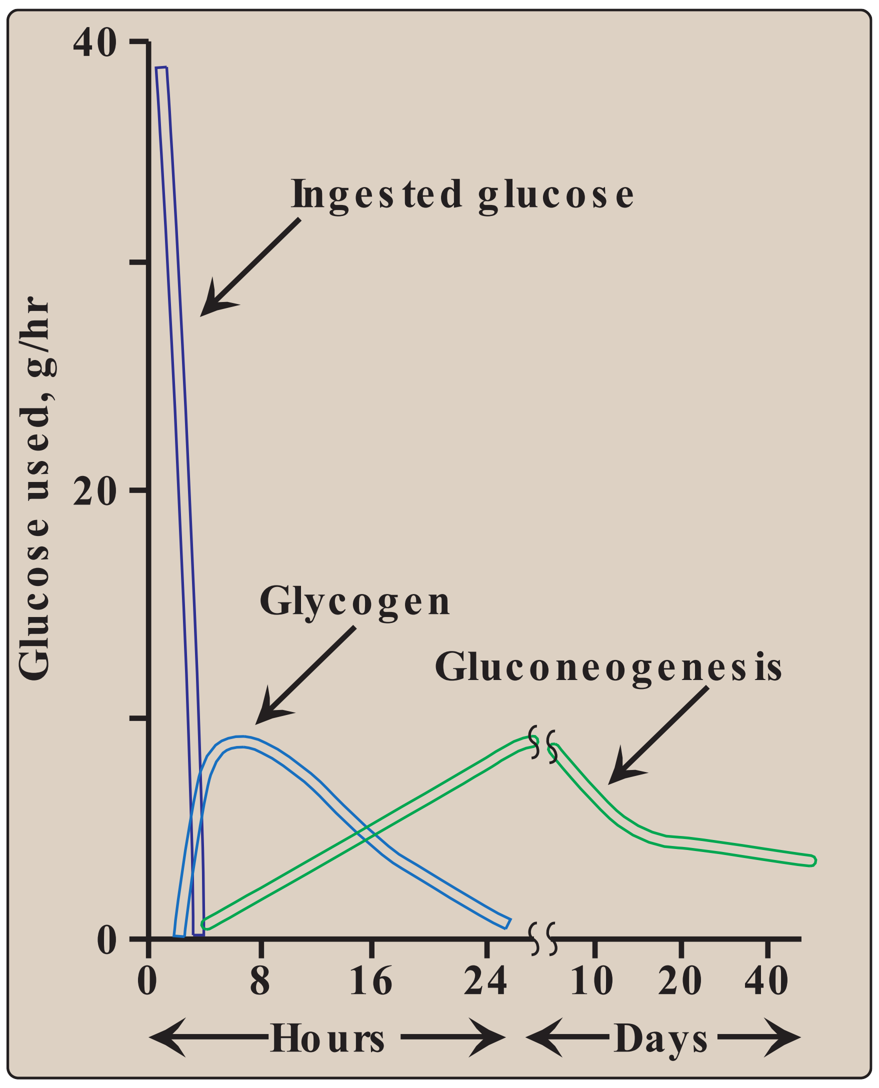


Figure 24.11
Sources of blood glucose after ingestion of 100 g of glucose. [Note: See Section B.2. for an explanation as to why gluconeogenesis declines.]

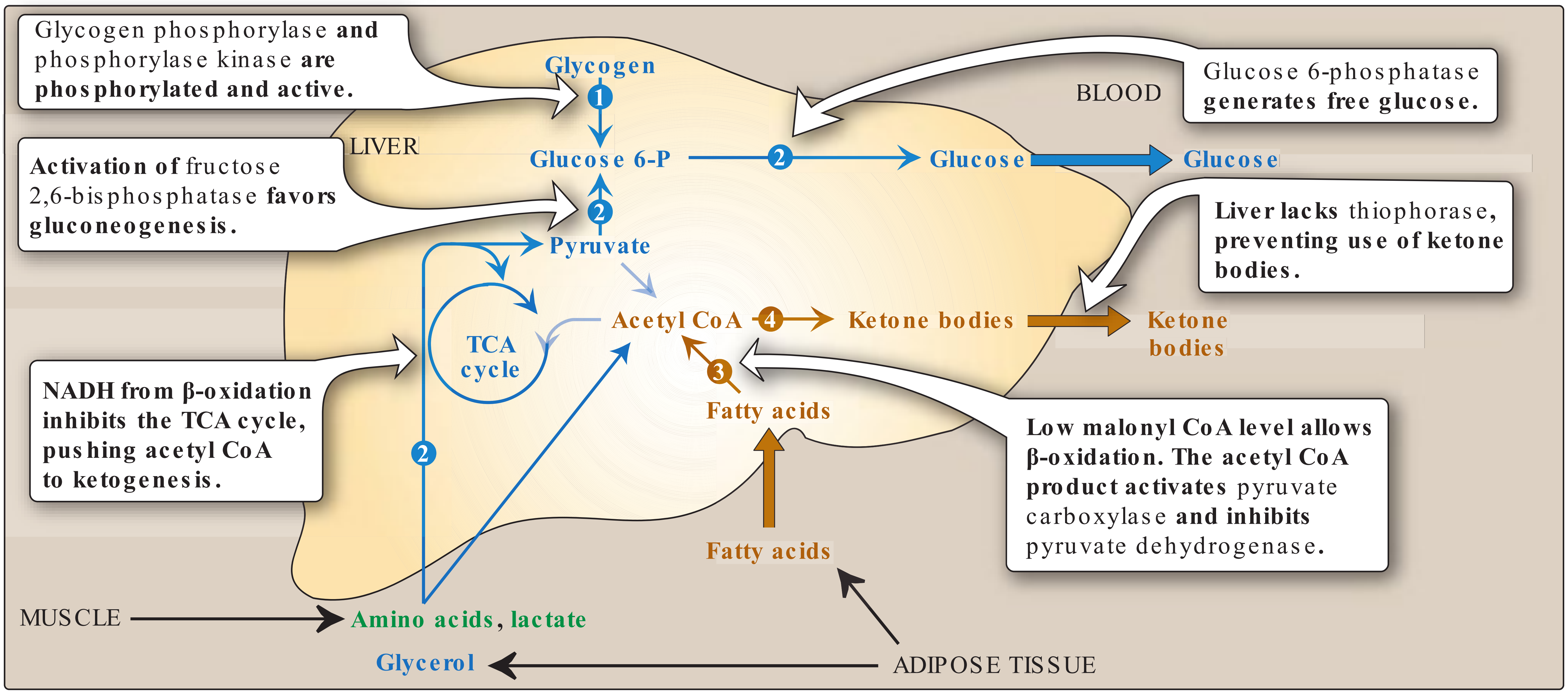

Glycogen phosphorylase and phosphorylase kinase are phosphorylated and active.
Glycogen
①
Glucose 6-P
②
Glucose
BLOOD
Glucose 6-phosphatase generates free glucose.
Glucose
LIVER
Activation of fructose 2,6-bisphosphatase favors gluconeogenesis.
②
Pyruvate
Liver lacks thiophorase, preventing use of ketone bodies.
TCA cycle
Acetyl CoA
④
Ketone bodies
Ketone bodies
③
Fatty acids
NADH from β-oxidation inhibits the TCA cycle, pushing acetyl CoA to ketogenesis.
②
Low malonyl CoA level allows β-oxidation. The acetyl CoA product activates pyruvate carboxylase and inhibits pyruvate dehydrogenase.
Fatty acids
MUSCLE
Amino acids, lactate
Glycerol
ADIPOSE TISSUE

Figure 24.12
Major metabolic pathways in liver during fasting. [Note: The numbers in circles, which appear both in the figure and in the corresponding citation in the text, indicate important metabolic pathways for carbohydrate or fat.] P = phosphate; CoA = coenzyme A; TCA = tricarboxylic acid; NADH = nicotinamide adenine dinucleotide.

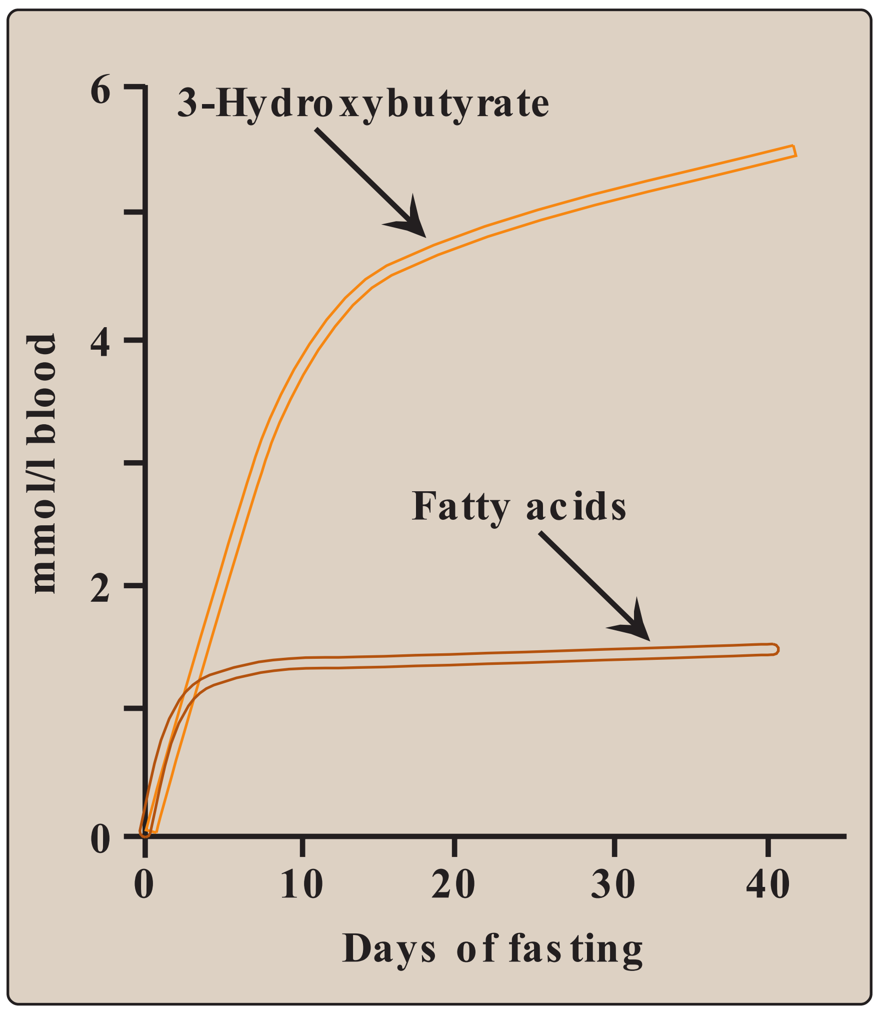


Figure 24.13
Concentrations of fatty acids and 3-hydroxybutyrate in the blood during fasting. [Note: 3-Hydroxybutyrate is made from the reduction of acetoacetate.]

by glucagon (see p. 122), begins 4–6 hours after the last meal and becomes fully active as stores of liver glycogen are depleted (see Figure 24.11). [Note: The decrease in fructose 2,6-bisphosphate simultaneously inhibits glycolysis at PFK-1 (see p. 99).]

B. Fat metabolism

1. **Increased fatty acid oxidation:** The oxidation of FAs obtained from TAG hydrolysis in adipose tissue is the major source of energy in hepatic tissue in the postabsorptive state (see Figure 24.12, □). The fall in malonyl CoA due to phosphorylation (inactivation) of ACC by AMPK removes the brake on CPT-1, allowing β-oxidation to occur (see p. 191). FA oxidation generates NADH, $FADH_2$, and acetyl CoA. The NADH inhibits the TCA cycle. The acetyl CoA is an allosteric activator of PC and an allosteric inhibitor of PDH, thereby favoring use of pyruvate in gluconeogenesis (see Figure 8.24). [Note: The acetyl CoA cannot be used as a substrate for gluconeogenesis, in part because the PDH reaction is irreversible.] Oxidation of NADH and $FADH_2$ coupled with oxidative phosphorylation supplies the energy required by the PC and PEPCK reactions of gluconeogenesis.

2. **Increased ketone body synthesis:** The liver is unique in being able to synthesize and release ketone bodies, primarily 3-hydroxybutyrate but also acetoacetate, for use as fuel by peripheral tissues (see p. 195) but not by the liver itself because liver lacks thiophorase. Ketogenesis, which starts during the first days of fasting (Figure 24.13), is favored when the concentration of acetyl CoA from FA oxidation exceeds the oxidative capacity of the TCA cycle. [Note:

Ketogenesis releases CoA, ensuring its availability for continued FA oxidation.] The availability of circulating water-soluble ketone bodies is important in fasting because they can be used for fuel by most tissues, including brain, once their level in the blood is sufficiently high. This reduces the need for gluconeogenesis from amino acid carbon skeletons, thus preserving essential protein (see Figure 24.11). Ketogenesis as part of the overall hepatic response to fasting is shown in Figure 24.12, □. [Note: Ketone bodies are organic acids and, when present at high concentrations, can cause ketoacidosis.]

IX. ADIPOSE TISSUE IN FASTING

A. Carbohydrate metabolism

Glucose transport by insulin-sensitive GLUT-4 into the adipocyte and its subsequent metabolism are depressed due to low levels of circulating insulin. This results in decreased TAG synthesis.

B. Fat metabolism

1. **Increased degradation of fat:** The PKA-mediated phosphorylation and activation of HSL (see p. 190) and subsequent hydrolysis of stored fat are enhanced by the elevated catecholamines norepinephrine and epinephrine. These hormones, which are released from the sympathetic nerve endings in adipose tissue and/or from the adrenal medulla, are physiologically important activators of HSL (Figure 24.14, □).

2. **Increased release of fatty acids:** FAs obtained from hydrolysis of stored TAG are primarily released into the blood (see Figure 24.14, □). Bound to albumin, they are transported to a variety of tissues for use as fuel. The glycerol produced from TAG degradation is used as a gluconeogenic precursor by the liver, which contains glycerol kinase. [Note: FA can also be oxidized to acetyl CoA, which can enter the TCA cycle, thereby producing energy for the adipocyte. They also can be re-esterified to glycerol 3-phosphate (from glyceroneogenesis, see p. 190), generating TAG and reducing plasma FA concentration.]

3. **Decreased uptake of fatty acids:** In fasting, LPL activity of adipose tissue is low. Consequently, circulating TAG of lipoproteins is not available to adipose tissue.

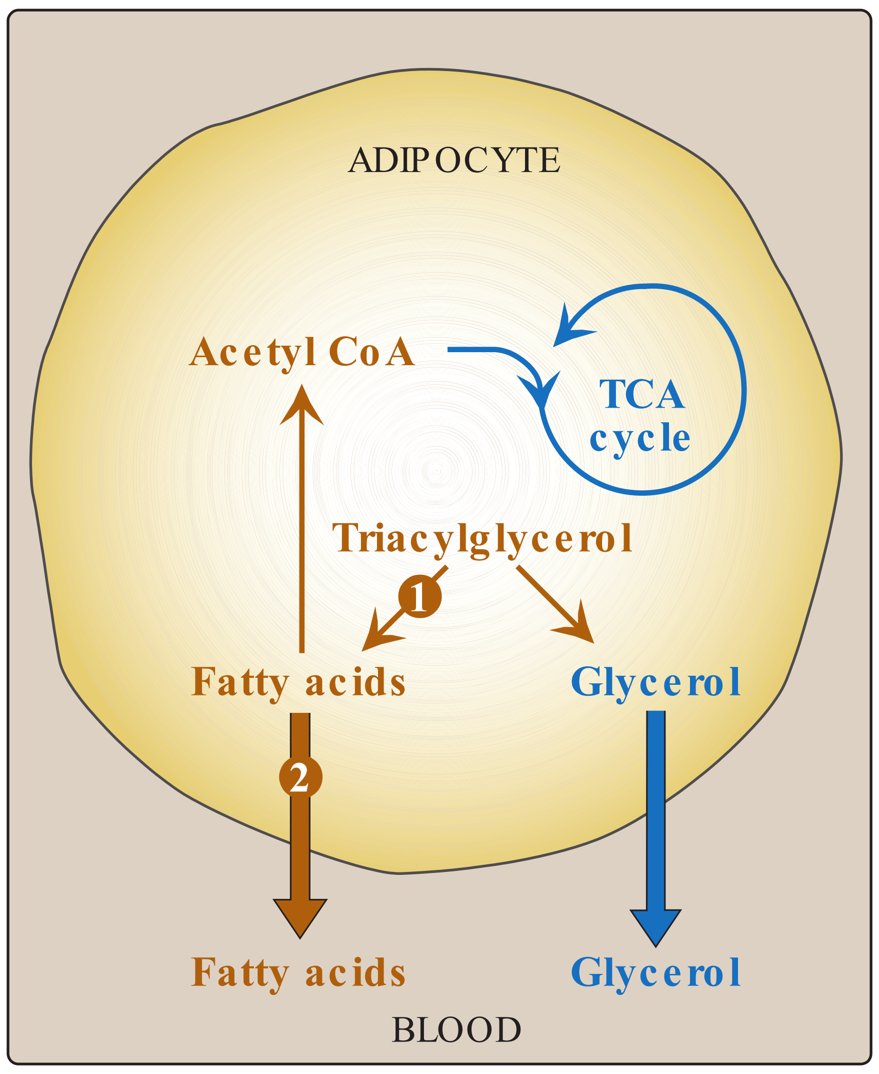


Figure 24.14
Major metabolic pathways in adipose tissue during fasting. [Note: The numbers in the circles, which appear both in the figure and in the corresponding citation in the text, indicate important pathways for fat metabolism.] CoA = coenzyme A; TCA = tricarboxylic acid.

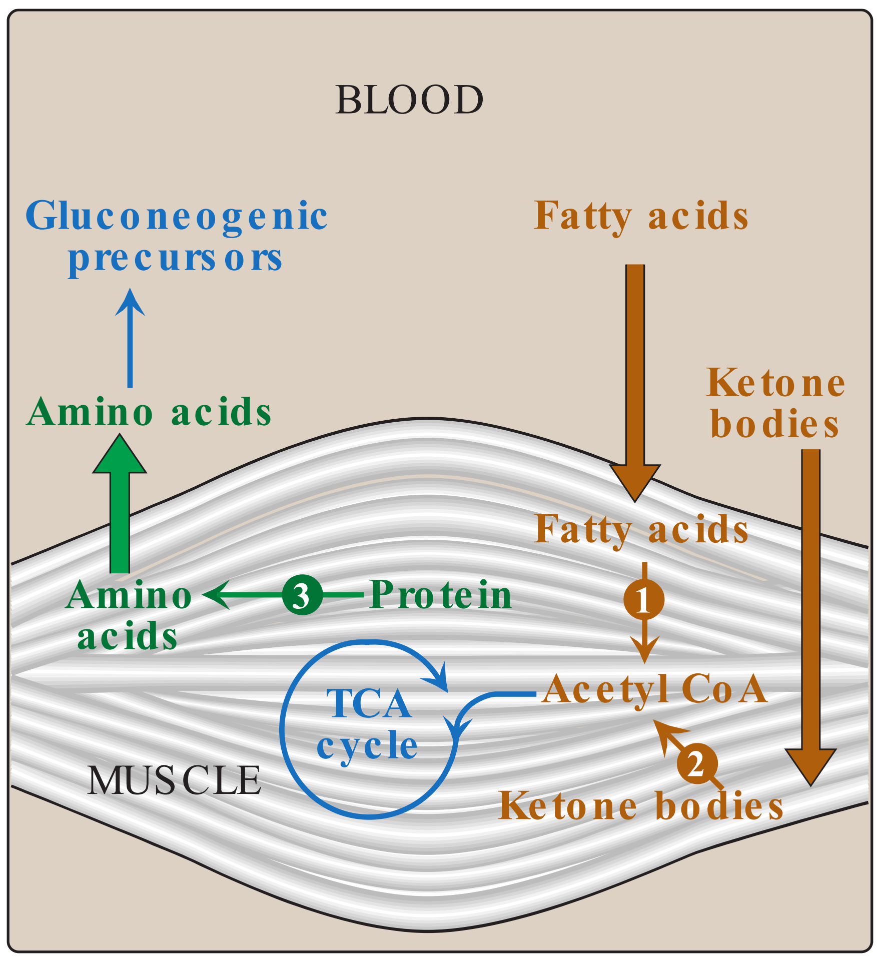


Figure 24.15
Major metabolic pathways in skeletal muscle during fasting. [Note: The numbers in the circles, which appear both in the figure and in the corresponding citation in the text, indicate important pathways for fat or protein metabolism.] CoA = coenzyme A; TCA = tricarboxylic acid.

X. RESTING SKELETAL MUSCLE IN FASTING

Resting muscle switches from glucose to FAs as its major fuel source in fasting. [Note: By contrast, exercising muscle initially uses its glycogen stores as a source of energy. During intense exercise, glucose 6-phosphate derived from glycogen is converted to lactate by anaerobic glycolysis (see p. 103). The lactate is used by liver for gluconeogenesis (Cori cycle; see p.118). As these glycogen reserves are depleted, free FAs provided by the mobilization of TAG from adipose tissue become the dominant energy source. The contraction-based rise in AMP activates AMPK that phosphorylates and inactivates the muscle isozyme of ACC, decreasing malonyl CoA and allowing FA oxidation (see p. 183).

A. Carbohydrate metabolism

Glucose transport into skeletal muscle cells via insulin-sensitive GLUT-4 (see p. 97) and subsequent glucose metabolism are depressed because of low levels of circulating insulin. Therefore, the glucose from hepatic gluconeogenesis is unavailable to muscle (and adipose tissue).

B. Lipid metabolism

During the first 2 weeks of fasting, muscle uses FA from adipose tissue and ketone bodies from the liver as fuels (Figure 24.15, □ and □). After about 3 weeks of fasting, muscle decreases its use of ketone bodies (thus sparing them for brain) and oxidizes FA almost exclusively. [Note: The acetyl CoA from FA oxidation indirectly inhibits PDH (by activation of PDH kinase) and spares pyruvate, which is transaminated to alanine and used by liver for gluconeogenesis (glucose–alanine cycle; see p. 253).]

C. Protein metabolism

During the first few days of fasting, there is a rapid breakdown of muscle protein, providing amino acids that are used by the liver for gluconeogenesis (see Figure 24.15, □). Because muscle does not have glucagon receptors, muscle proteolysis is initiated by a fall in insulin and sustained by a rise in glucocorticoids. [Note: Alanine and glutamine are quantitatively the most important gluconeogenic amino acids released from muscle. They are produced by the catabolism of BCAAs (see p. 267).] The glutamine is used as a fuel by enterocytes, for example, which send out alanine that is used in hepatic gluconeogenesis. In the second week of fasting, the rate of muscle proteolysis decreases, paralleling a decline in the need for glucose as a fuel for the brain, which has begun using ketone bodies as a source of energy.

XI. BRAIN IN FASTING

During the early days of fasting, the brain continues to use only glucose as a fuel (Figure 24.16, □). Blood glucose is maintained by hepatic gluconeogenesis from glucogenic precursors, such as amino acids from proteolysis and glycerol from lipolysis. In prolonged fasting (beyond 2–3 weeks), plasma ketone bodies (see Figure 24.12) reach significantly elevated levels and replace glucose as the primary fuel for the brain (see Figure 24.16, □, and Figure 24.17). This reduces the need for protein catabolism for gluconeogenesis: ketone bodies spare glucose and, thus, muscle protein. [Note: As the duration of a fast extends from overnight to days to weeks, blood glucose levels initially drop and then are maintained at the lower level (65–70 mg/dl).] The metabolic changes that occur during fasting ensure that all tissues have an adequate supply of fuel molecules. The response of the major tissues involved in energy metabolism during fasting is summarized in Figure 24.19 (see below).

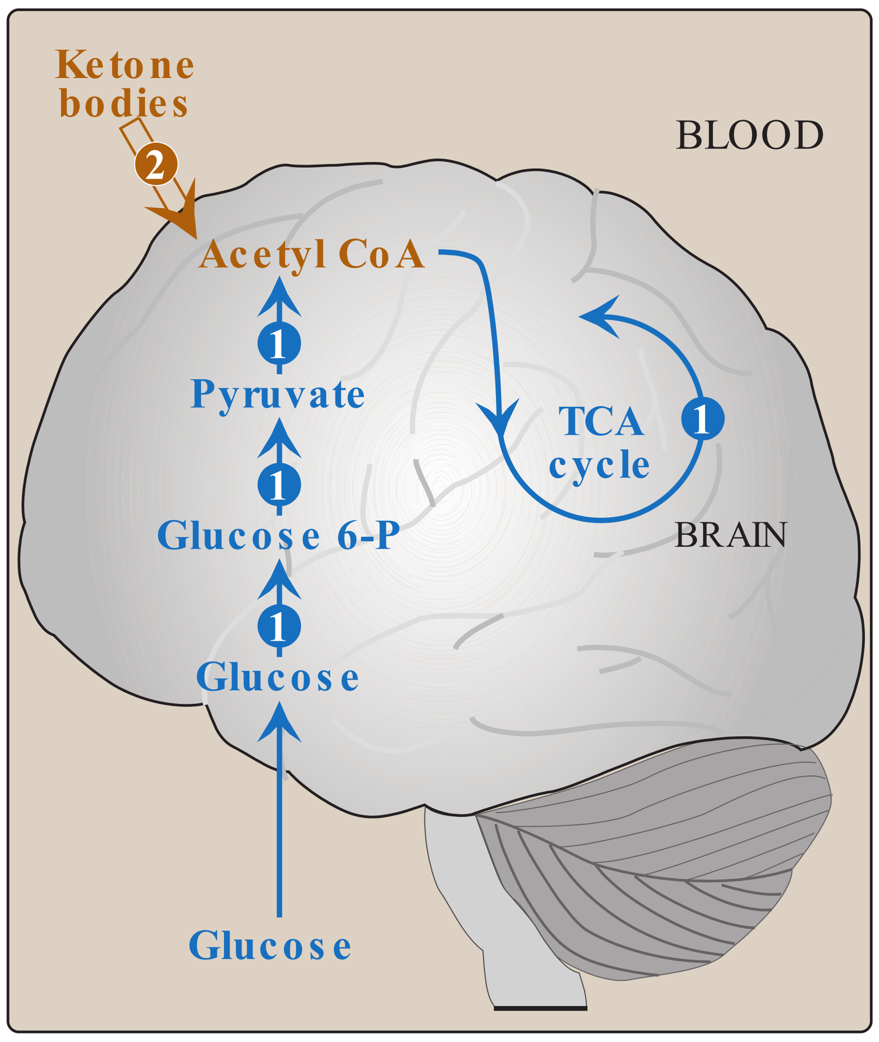


Figure 24.16
Major metabolic pathways in the brain during fasting. [Note: The numbers in the circles, which appear both in the figure and in the corresponding citation in the text, indicate important pathways for metabolism of fat or carbohydrates.] CoA = coenzyme A; TCA = tricarboxylic acid; P = phosphate.

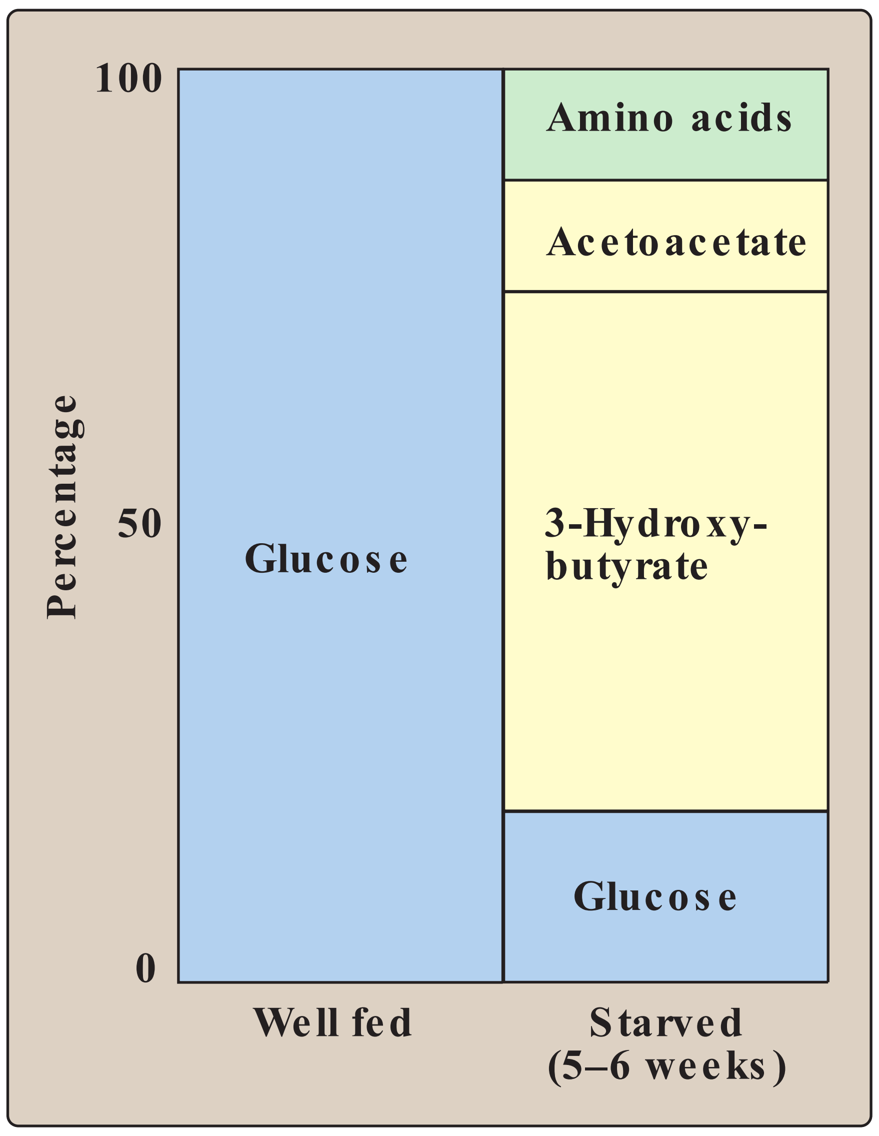


Figure 24.17
Fuel sources used by the brain to meet energy needs in the well fed and starved states.

XII. KIDNEY IN LONG-TERM FASTING

As fasting continues into early starvation and beyond, the kidney plays important roles. The kidney expresses the enzymes of gluconeogenesis, including glucose 6-phosphatase, and in late fasting about 50% of gluconeogenesis occurs here. [Note: A portion of this glucose is used by the kidney itself.] The kidney also provides compensation for the acidosis that accompanies the increased production of ketone bodies (organic acids). The glutamine released from the muscle's metabolism of BCAAs is taken up by the kidney and acted upon by renal glutaminase and glutamate dehydrogenase (see p. 256), producing α-ketoglutarate that can be used as a substrate for gluconeogenesis, plus ammonia (NH_3). The NH_3 picks up protons from ketone body dissociation and is excreted in the urine as ammonium (NH_4^+), thereby decreasing the acid load in the body (Figure 24.18). In long-term fasting, then, there is a switch from nitrogen disposal in the form of urea to disposal in the form of ammonia. [Note: As ketone body concentration rises, enterocytes, typically consumers of glutamine, become consumers of ketone bodies. This allows more glutamine to be available to the kidney.]

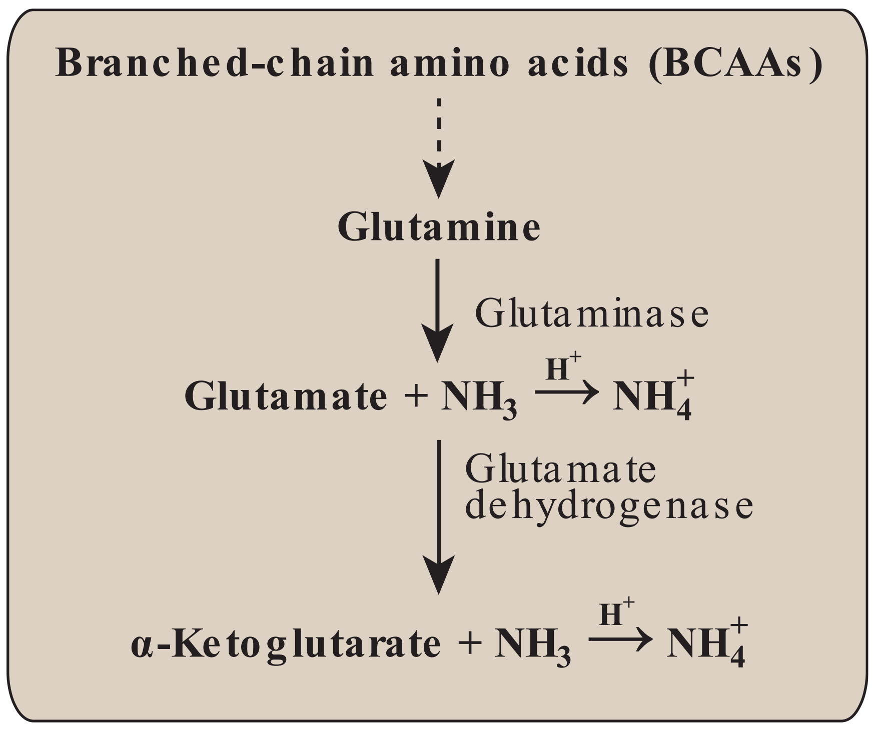
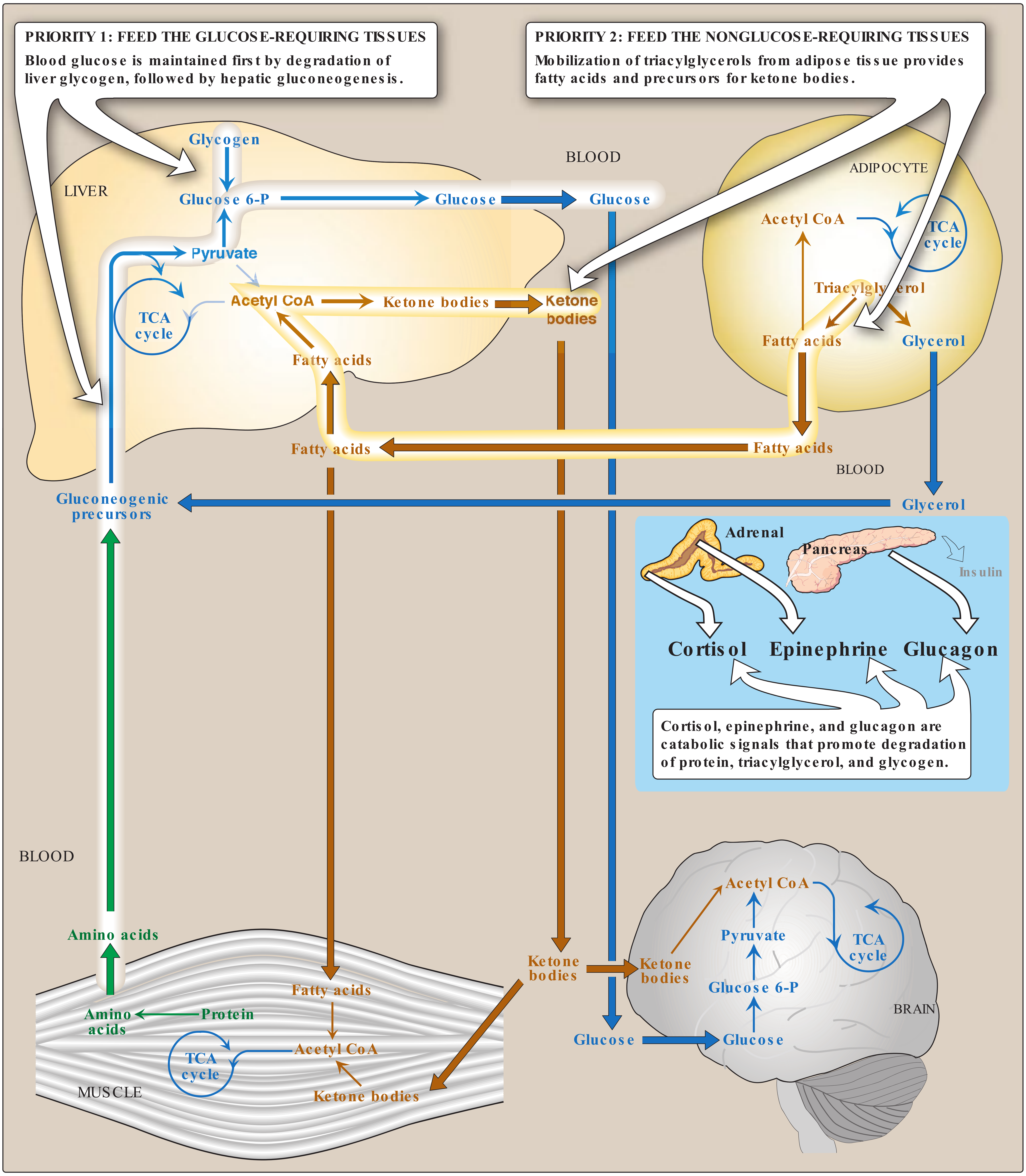

Branched-chain amino acids (BCAAs)
Glutamine
Glutaminase
Glutamate + $NH_3 \xrightarrow{H^+} NH_4^+$
Glutamate dehydrogenase
α-Ketoglutarate + $NH_3 \xrightarrow{H^+} NH_4^+$

Figure 24.18
Use of glutamine from BCAA catabolism in muscle to generate ammonia (NH_3) used for the excretion of protons (H^+) as ammonium (NH_4^+) in kidney.

XIII. CHAPTER SUMMARY

The flow of intermediates through metabolic pathways is controlled by four mechanisms: 1) the availability of substrates, 2) allosteric activation and inhibition of enzymes, 3) covalent modification of enzymes, and 4) induction-repression of enzyme synthesis. In the absorptive state, the 2–4-hour period after ingestion of a meal, these regulatory mechanisms ensure that available nutrients are captured as **glycogen**, **triacylglycerol** (**TAG**), and **protein** (Figure 24.20). During this interval, transient increases in plasma glucose, amino acids, and TAG occur, the last primarily as components of chylomicrons synthesized by the intestinal mucosal cells. The **pancreas** responds to the elevated levels of glucose with an **increased secretion of insulin** and a **decreased secretion of glucagon** by the **pancreas**. The elevated insulin-to-glucagon ratio and the ready availability of circulating substrates make the absorptive state an **anabolic period** during which virtually all tissues use **glucose** as a fuel. In addition, the **liver** replenishes its **glycogen** stores, replaces any needed **hepatic proteins**, and increases **TAG** synthesis. The latter are packaged in **very-low-density lipoproteins**, which are exported to the peripheral tissues. **Adipose tissue** increases **TAG** synthesis and storage, whereas **muscle** increases **protein** synthesis to replace protein degraded since the previous meal. In the fed state, the **brain** uses glucose exclusively as a fuel. In the **absence of food**, plasma levels of glucose, amino acids, and TAG fall, triggering a **decline** in **insulin secretion** and an **increase in glucagon** and **epinephrine release**. The decreased insulin/counterregulatory hormone ratio and the decreased availability of circulating substrates make the fasting state a **catabolic period**. This sets into motion an **exchange of substrates** among liver, adipose tissue, skeletal muscle, and brain that is guided by two priorities: 1) the need to maintain adequate plasma levels of glucose to sustain energy metabolism of the brain and other glucose-requiring tissues and 2) the need to mobilize fatty acids (FAs) from adipose tissue and release ketone bodies from liver to supply energy to other tissues. To accomplish these goals, the **liver** degrades **glycogen** and initiates **gluconeogenesis**, using **increased fatty acid oxidation** as a source of the energy and reducing equivalents needed for gluconeogenesis and to supply the acetyl coenzyme A building blocks for **ketogenesis**. The **adipose tissue** degrades stored **TAG**, thus providing **FAs** and **glycerol** to the liver. The **muscle** can also use **FAs** as fuel as well as **ketone bodies** supplied by the liver. Muscle **protein** is **degraded** to supply **amino acids** for the liver to use in gluconeogenesis, but deceases as ketone bodies increase. The **brain** can use both **glucose** and **ketone bodies** as fuels. From late fasting into starvation, the **kidneys** play important roles by **synthesizing glucose** and **excreting the protons** from ketone body dissociation as ammonium (**NH_4^+**).



PRIORITY 1: FEED THE GLUCOSE-REQUIRING TISSUES

Blood glucose is maintained first by degradation of liver glycogen, followed by hepatic gluconeogenesis.

PRIORITY 2: FEED THE NONGLUCOSE-REQUIRING TISSUES

Mobilization of triacylglycerols from adipose tissue provides fatty acids and precursors for ketone bodies.

Cortisol, epinephrine, and glucagon are catabolic signals that promote degradation of protein, triacylglycerol, and glycogen.

LIVER
Glycogen
Glucose 6-P
Glucose
Pyruvate
TCA cycle
Acetyl CoA
Ketone bodies
Fatty acids
Fatty acids
BLOOD
Glucose
Ketone bodies
ADIPOCYTE
Acetyl CoA
TCA cycle
Triacylglycerol
Fatty acids
Glycerol
Fatty acids
BLOOD
Glycerol
Gluconeogenic precursors
Adrenal
Pancreas
Insulin
Cortisol
Epinephrine
Glucagon
BLOOD
Amino acids
Amino acids
Protein
TCA cycle
Fatty acids
Acetyl CoA
Ketone bodies
MUSCLE
Ketone bodies
Glucose
Ketone bodies
Acetyl CoA
Pyruvate
TCA cycle
Glucose 6-P
Glucose
BRAIN

Figure 24.19

Intertissue relationships during starvation and the hormonal signals that promote them. P = phosphate; TCA = tricarboxylic acid; CoA = coenzyme A.

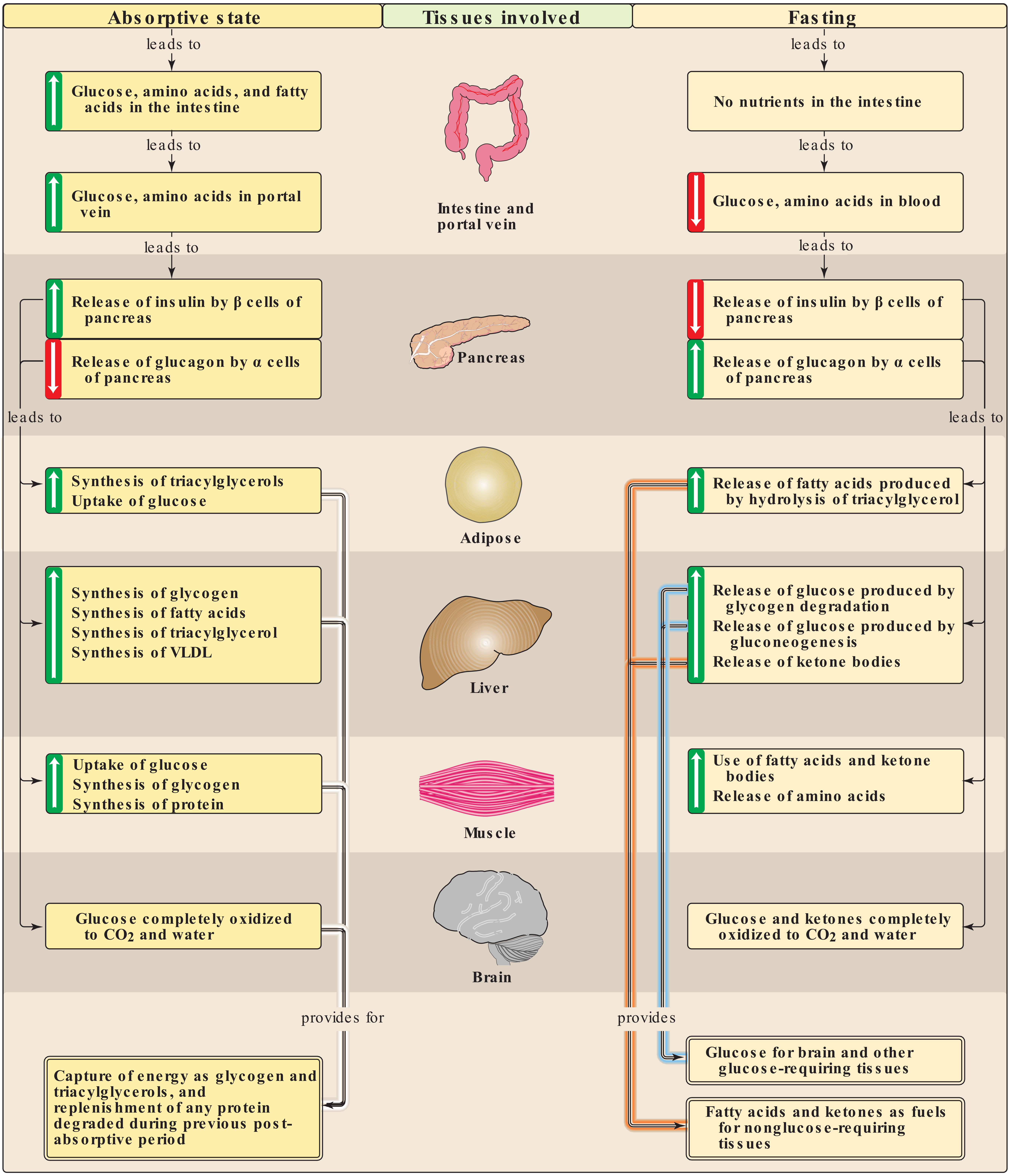

Absorptive state
Tissues involved
Fasting
leads to
↑ Glucose, amino acids, and fatty acids in the intestine
leads to
↑ Glucose, amino acids in portal vein
leads to
↑ Release of insulin by β cells of pancreas
↓ Release of glucagon by α cells of pancreas
leads to
↑ Synthesis of triacylglycerols Uptake of glucose
↑ Synthesis of glycogen Synthesis of fatty acids Synthesis of triacylglycerol Synthesis of VLDL
↑ Uptake of glucose Synthesis of glycogen Synthesis of protein
Glucose completely oxidized to CO2 and water
provides for
Capture of energy as glycogen and triacylglycerols, and replenishment of any protein degraded during previous post-absorptive period
Intestine and portal vein
Pancreas
Adipose
Liver
Muscle
Brain
leads to
No nutrients in the intestine
leads to
↓ Glucose, amino acids in blood
leads to
↓ Release of insulin by β cells of pancreas
↑ Release of glucagon by α cells of pancreas
leads to
↑ Release of fatty acids produced by hydrolysis of triacylglycerol
↑ Release of glucose produced by glycogen degradation Release of glucose produced by gluconeogenesis Release of ketone bodies
↑ Use of fatty acids and ketone bodies Release of amino acids
Glucose and ketones completely oxidized to CO2 and water
provides
Glucose for brain and other glucose-requiring tissues
Fatty acids and ketones as fuels for nonglucose-requiring tissues

Figure 24.20
Key concept map for feed–fast cycle. VLDL = very-low-density lipoprotein.

Diabetes Mellitus

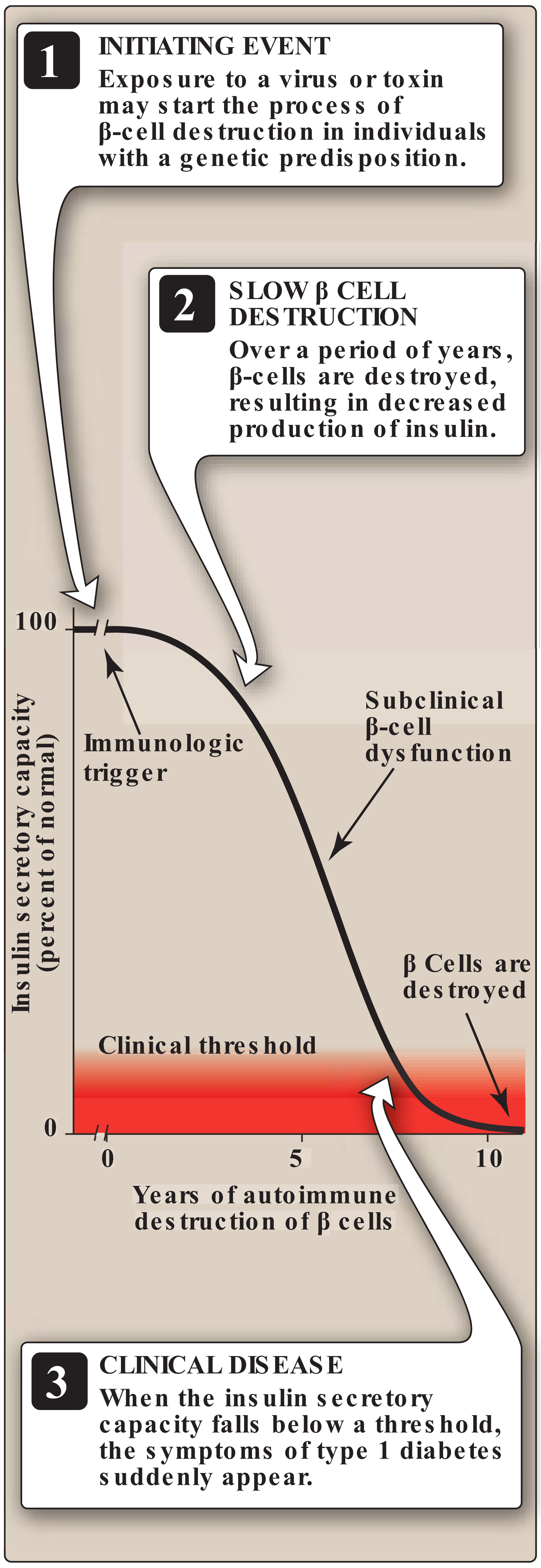
25

I. OVERVIEW OF DIABETES MELLITUS

Diabetes mellitus ("diabetes") is not one disease, but rather is a heterogeneous group of multifactorial, polygenic syndromes characterized by an elevated fasting blood glucose (FBG) caused by a relative or absolute deficiency in insulin. Nearly 26 million people in the United States (about 8% of the population) have diabetes. Of this number, approximately 7 million are as yet undiagnosed. Diabetes is the leading cause of adult blindness and amputation and a major cause of renal failure, nerve damage, heart attacks, and strokes. Most cases of diabetes mellitus can be separated into two groups (Figure 25.1), type 1 ([T1D] formerly called insulin-dependent diabetes mellitus) and type 2 ([T2D] formerly called noninsulin-dependent diabetes mellitus). The incidence and prevalence of T2D is increasing because of the aging of the U.S. population and the increasing prevalence of obesity and sedentary lifestyles (see p. 349). The increase in children with T2D is particularly disturbing.

	Type 1 Diabetes	Type 2 Diabetes
AGE OF ONSET	Usually during childhood or puberty; symptoms develop rapidly	Frequently after age 35 years; symptoms develop gradually
NUTRITIONAL STATUS AT TIME OF DISEASE ONSET	Frequently undernourished	Obesity usually present
PREVALENCE	<10% of diagnosed diabetics	>90% of diagnosed diabetics
GENETIC PREDISPOSITION	Moderate	Very strong
DEFECT OR DEFICIENCY	β Cells are destroyed, eliminating production of insulin	Insulin resistance combined with inability of β cells to produce appropriate quantities of insulin
FREQUENCY OF KETOSIS	Common	Rare
PLASMA INSULIN	Low to absent	High early in disease; low to absent in disease of long duration
ACUTE COMPLICATIONS	Ketoacidosis	Hyperosmolar hyperglycemic state
RESPONSE TO ORAL HYPOGLYCEMIC DRUGS	Unresponsive	Responsive
TREATMENT	Insulin is always necessary	Diet, exercise, oral hypoglycemic drugs, insulin (may or may not be necessary); reduction of risk factors (weight reduction, smoking cessation, blood pressure control, treatment of dyslipidemia) is essential to therapy

Figure 25.1
Comparison of type 1 and type 2 diabetes mellitus. [Note: The name of the disease reflects the clinical presentation of copious amounts of glucose-containing urine and is derived from the Greek word for siphon (diabetes) and the Latin word for honey-sweet (mellitus).]



1 INITIATING EVENT
Exposure to a virus or toxin may start the process of β-cell destruction in individuals with a genetic predisposition.

2 SLOW β CELL DESTRUCTION
Over a period of years, β-cells are destroyed, resulting in decreased production of insulin.

3 CLINICAL DISEASE
When the insulin secretory capacity falls below a threshold, the symptoms of type 1 diabetes suddenly appear.

100
0
Insulin secretory capacity (percent of normal)
Immunologic trigger
Subclinical β-cell dysfunction
β Cells are destroyed
Clinical threshold
0
5
10
Years of autoimmune destruction of β cells

Figure 25.2
Insulin secretory capacity during onset of type 1 diabetes. [Note: Rate of autoimmune destruction of β cells may be faster or slower than shown.]

II. TYPE 1 DIABETES

Persons with T1D constitute less than 10% of the nearly 20 million known diabetics in the United States. The disease is characterized by an absolute deficiency of insulin caused by an autoimmune attack on the β cells of the pancreas. In T1D, the islets of Langerhans become infiltrated with activated T lymphocytes, leading to a condition called insulitis. Over a period of years, this autoimmune attack on the β cells leads to gradual depletion of the β-cell population (Figure 25.2). However, symptoms appear abruptly when 80%–90% of the β cells have been destroyed. At this point, the pancreas fails to respond adequately to ingestion of glucose, and insulin therapy is required to restore metabolic control and prevent life-threatening ketoacidosis. β-Cell destruction requires both a stimulus from the environment (such as a viral infection) and a genetic determinant that allows the β cells to be recognized as "nonself." [Note: Among monozygotic (identical) twins, if one sibling develops T1D, the other twin has only a 30%–50% chance of developing the disease. This contrasts with T2D (see p. 341), in which the genetic influence is stronger and, in virtually all monozygotic twinships, the disease eventually develops in both individuals.]

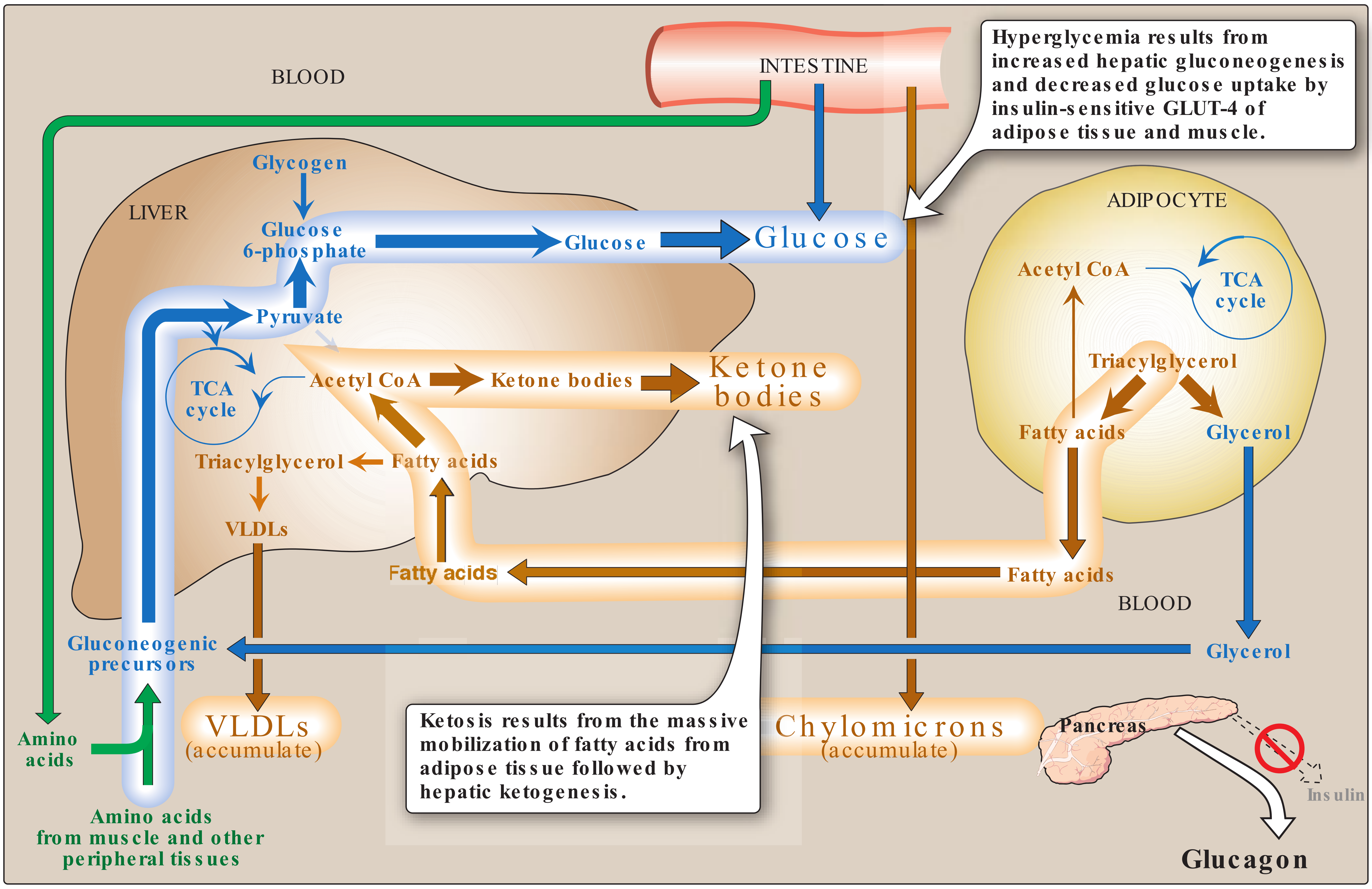
A. Diagnosis of type 1 diabetes

The onset of T1D is typically during childhood or puberty, and symptoms develop suddenly. Patients with T1D can usually be recognized by the abrupt appearance of polyuria (frequent urination), polydipsia (excessive thirst), and polyphagia (excessive hunger), often triggered by physiologic stress such as an illness. These symptoms are usually accompanied by fatigue and weight loss. The diagnosis is confirmed by a glycosylated hemoglobin (see p. 340) concentration ≥ 6.5 mg/dl (normal is less than 5.7), or a FBG ≥ 126 mg/dl (normal is 70–99). [Note: A FBG of 100–125 mg/dl is categorized as an impaired FBG. Individuals with impaired FBG are considered "prediabetic" and are at increased risk for developing T2D.] Fasting is defined as no caloric intake for at least 8 hours. Diagnosis can also be made on the basis of a nonfasting (random) blood glucose level greater than 200 mg/dl in an individual with symptoms of hyperglycemia. [Note: The oral glucose tolerance test, in which blood glucose is measured 2 hours after ingestion of a solution containing 75 g of glucose, also is used but is less convenient. It is most typically used to identify pregnant women with gestational diabetes (see p. 342).]

When blood glucose is greater than 180 mg/dl, the ability of the kidneys to reclaim glucose is impaired. This results in glucose "spilling" into the urine. The loss of glucose is accompanied by the loss of water, resulting in the characteristic polyuria (with dehydration) and polydipsia of diabetes.

B. Metabolic changes in type 1 diabetes

The metabolic abnormalities of T1D mellitus result from a deficiency of insulin that profoundly affects metabolism in three tissues: liver, muscle, and adipose (Figure 25.3).


BLOOD
INTESTINE
LIVER
Glycogen
Glucose 6-phosphate
Glucose
Glucose
Pyruvate
TCA cycle
Acetyl CoA
Ketone bodies
Ketone bodies
Triacylglycerol
Fatty acids
VLDLs
Fatty acids
Fatty acids
ADIPOCYTE
Acetyl CoA
TCA cycle
Triacylglycerol
Fatty acids
Glycerol
BLOOD
Glycerol
Gluconeogenic precursors
Amino acids
VLDLs (accumulate)
Chylomicrons (accumulate)
Pancreas
Insulin
Glucagon
Amino acids from muscle and other peripheral tissues

Hyperglycemia results from increased hepatic gluconeogenesis and decreased glucose uptake by insulin-sensitive GLUT-4 of adipose tissue and muscle.

Ketosis results from the massive mobilization of fatty acids from adipose tissue followed by hepatic ketogenesis.

Figure 25.3
Intertissue relationships in type 1 diabetes. TCA = tricarboxylic acid; CoA = coenzyme A; VLDLs = very-low-density lipoproteins; GLUT = glucose transporter.

1. **Hyperglycemia and ketoacidosis:** Elevated levels of blood glucose and ketone bodies are the hallmarks of untreated T1D (see Figure 25.3). Hyperglycemia is caused by increased hepatic production of glucose via gluconeogenesis, combined with diminished peripheral utilization (muscle and adipose tissue have the insulin-sensitive glucose transporter GLUT-4; see p. 97). Ketosis results from increased mobilization of fatty acids (FAs) from adipose tissue, combined with accelerated hepatic FA β-oxidation and synthesis of 3-hydroxybutyrate and acetoacetate. [Note: Acetyl coenzyme A from β-oxidation is the substrate for ketogenesis and the allosteric activator of pyruvate carboxylase, a gluconeogenic enzyme.] Diabetic ketoacidosis (DKA), a type of metabolic acidosis, occurs in 25%–40% of those newly diagnosed with T1D and may recur if the patient becomes ill (most commonly with an infection) or does not comply with therapy. DKA is treated by replacing fluid and electrolytes and administering short-acting insulin to gradually correct hyperglycemia without precipitating hypoglycemia.

2. **Hypertriacylglycerolemia:** Not all of the FAs flooding the liver can be disposed of through oxidation or ketone body synthesis. These excess fatty acids are converted to triacylglycerol (TAG), which is packaged and secreted in very-low-density lipoproteins ([VLDLs] see p. 231). Chylomicrons are synthesized from

dietary lipids by the intestinal mucosal cells following a meal (see p. 178). Because lipoprotein degradation catalyzed by lipoprotein lipase in the capillary beds of adipose tissue (see. p. 228) is low in diabetics (synthesis of the enzyme is decreased when insulin levels are low), the plasma chylomicron and VLDL levels are elevated, resulting in hypertriacylglycerolemia (see Figure 25.3).

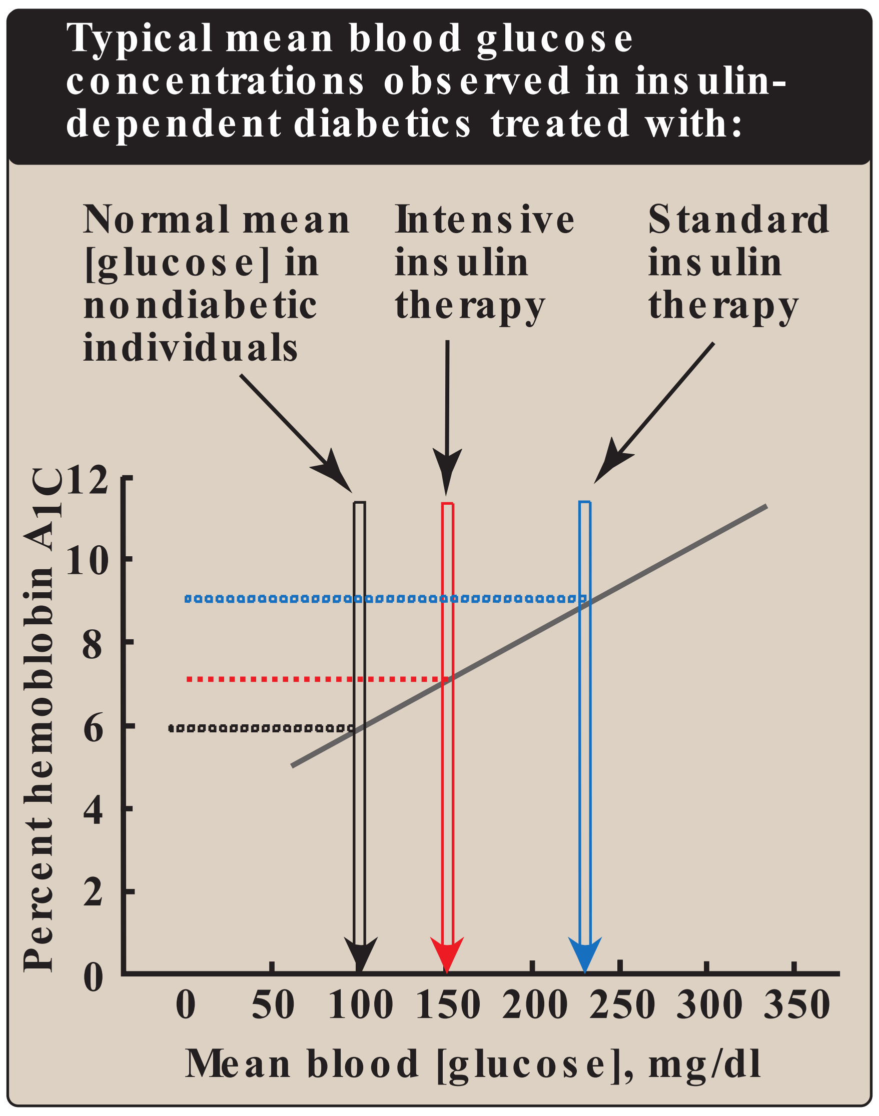


Figure 25.4
Correlation between mean blood glucose and hemoglobin A_{1C} in patients with type 1 diabetes.

C. Treatment of type 1 diabetes

Individuals with T1D must rely on exogenous insulin delivered subcutaneously either by periodic injection or continuous pump-assisted infusion to control the hyperglycemia and ketoacidosis. Two therapeutic regimens are currently in use, standard and intensive insulin treatment.

1. **Standard treatment versus intensive treatment:** Standard treatment typically consists of one or two daily injections of recombinant human insulin. Mean blood glucose levels obtained are typically in the 225–275 mg/dl range, with a glycosylated hemoglobin (HbA_{1c}) level (see p. 34) of 8%–9% of the total hemoglobin (blue arrow in Figure 25.4). [Note: The rate of formation of HbA_{1c} is proportional to the average blood glucose concentration over the previous 3 months. Thus, HbA_{1c} provides a measure of how well treatment has normalized blood glucose in the diabetic patient over that time.] In contrast to standard therapy, intensive treatment seeks to more closely normalize blood glucose through more frequent monitoring and subsequent injections of insulin, typically three or more times a day. Mean blood glucose levels of 150 mg/dl can be achieved, with HbA_{1c} approximately 7% of the total hemoglobin (see red arrow in Figure 25.4). [Note: Normal mean blood glucose is approximately 100 mg/dl, and HbA_{1c} is 6% or less (see black arrow in Figure 25.4).] Therefore, normalization of glucose values (euglycemia) is not achieved even in intensively treated patients. Nonetheless, patients on intensive therapy show a 50% or more reduction in the long-term microvascular complications of diabetes (that is, retinopathy, nephropathy, and neuropathy) compared with patients receiving standard care. This confirms that the complications of diabetes are related to an elevation of plasma glucose.

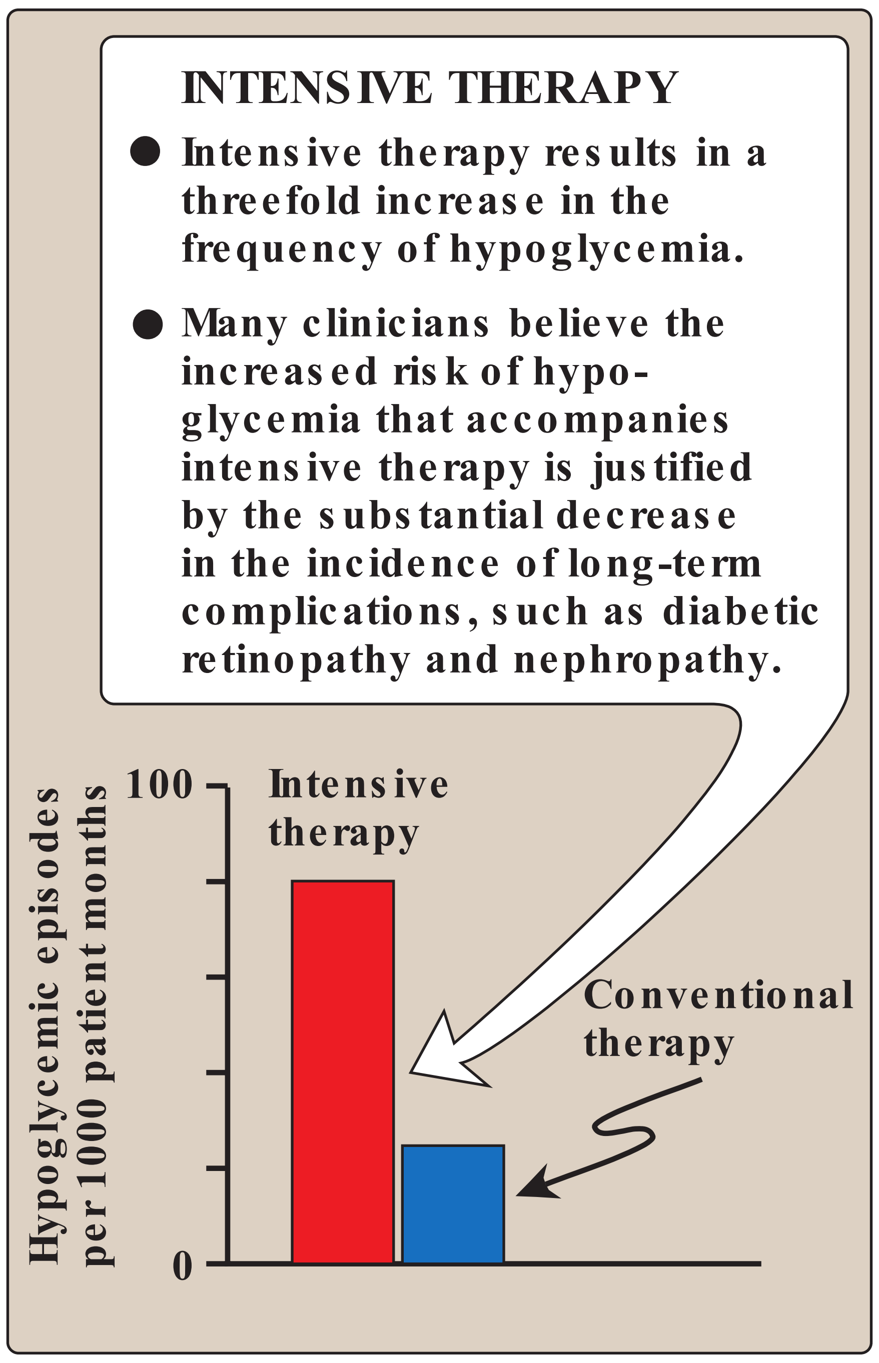


Figure 25.5
Effect of tight glucose control on hypoglycemic episodes in a population of patients on intensive therapy or conventional therapy.

2. **Hypoglycemia in type 1 diabetes:** One of the therapeutic goals in cases of diabetes is to decrease blood glucose levels in an effort to minimize the development of long-term complications of the disease (see p. 344 for a discussion of the chronic complications of diabetes). However, appropriate dosage of insulin is difficult to achieve. Hypoglycemia caused by excess insulin is the most common complication of insulin therapy, occurring in over 90% of patients. The frequency of hypoglycemic episodes, coma, and seizures is particularly high with intensive treatment regimens designed to achieve tight control of blood glucose (Figure 25.5). Recall that in normal individuals, hypoglycemia triggers a compensatory secretion of counterregulatory hormones, most notably glucagon and epinephrine, which promote hepatic production of glucose. However, patients with T1D also develop a deficiency of glucagon secretion. This defect occurs early in the disease and is almost universally present 4 years after diagnosis. These patients,

therefore, rely on epinephrine secretion to prevent severe hypoglycemia. However, as the disease progresses, T1D patients show diabetic autonomic neuropathy and impaired ability to secrete epinephrine in response to hypoglycemia. The combined deficiency of glucagon and epinephrine secretion creates a symptom-free condition sometimes called "hypoglycemia unawareness." Thus, patients with long-standing T1D are particularly vulnerable to hypoglycemia. Hypoglycemia can also be caused by strenuous exercise. Exercise promotes glucose uptake into muscle and decreases the need for exogenous insulin. Patients are advised, therefore, to check blood glucose levels before or after intensive exercise to prevent or abort hypoglycemia.

3. **Contraindications for tight control:** Children are not put on a program of tight control of blood glucose before age 8 years because of the risk that episodes of hypoglycemia may adversely affect brain development. Elderly people typically do not go on tight control because hypoglycemia can cause strokes and heart attacks in this population. Also, the major goal of tight control is to prevent complications many years later. Tight control, then, is most worthwhile for otherwise healthy people who can expect to live at least 10 more years. [Note: For most nonpregnant adults with diabetes, the individual treatment strategies and goals are based on the duration of diabetes, age/life expectancy, and known comorbid conditions.]

III. TYPE 2 DIABETES

T2D is the most common form of the disease, afflicting over 90% of the diabetic population in the United States. [Note: American Indians, Alaskan Natives, Hispanic and Latino Americans, African Americans, and Asian Americans have the highest prevalence.] Typically, T2D develops gradually without obvious symptoms. The disease is often detected by routine screening tests. However, many individuals with T2D have symptoms of polyuria and polydipsia of several weeks' duration. Polyphagia may be present but is less common. Patients with T2D have a combination of insulin resistance and dysfunctional β cells (Figure 25.6) but do not require insulin to sustain life, although insulin eventually will be required to control hyperglycemia and keep HbA_{1c} below 7% in over 90% of patients. The metabolic alterations observed in T2D are milder than those described for type 1, in part, because insulin secretion in T2D, although inadequate, does restrain ketogenesis and blunts the development of DKA. (Recall that insulin suppresses the release of glucagon.) Diagnosis is based on the presence of hyperglycemia as described above. The pathogenesis does not involve viruses or autoimmune antibodies and is not completely understood. [Note: An acute complication of T2D in the elderly is a hyperosmolar hyperglycemic state characterized by severe hyperglycemia and dehydration and altered mental status.]

> T2D is characterized by hyperglycemia; insulin resistance; impaired insulin secretion; and, ultimately, β-cell failure. The eventual need for insulin therapy has eliminated the designation of T2D as "noninsulin-dependent" diabetes.

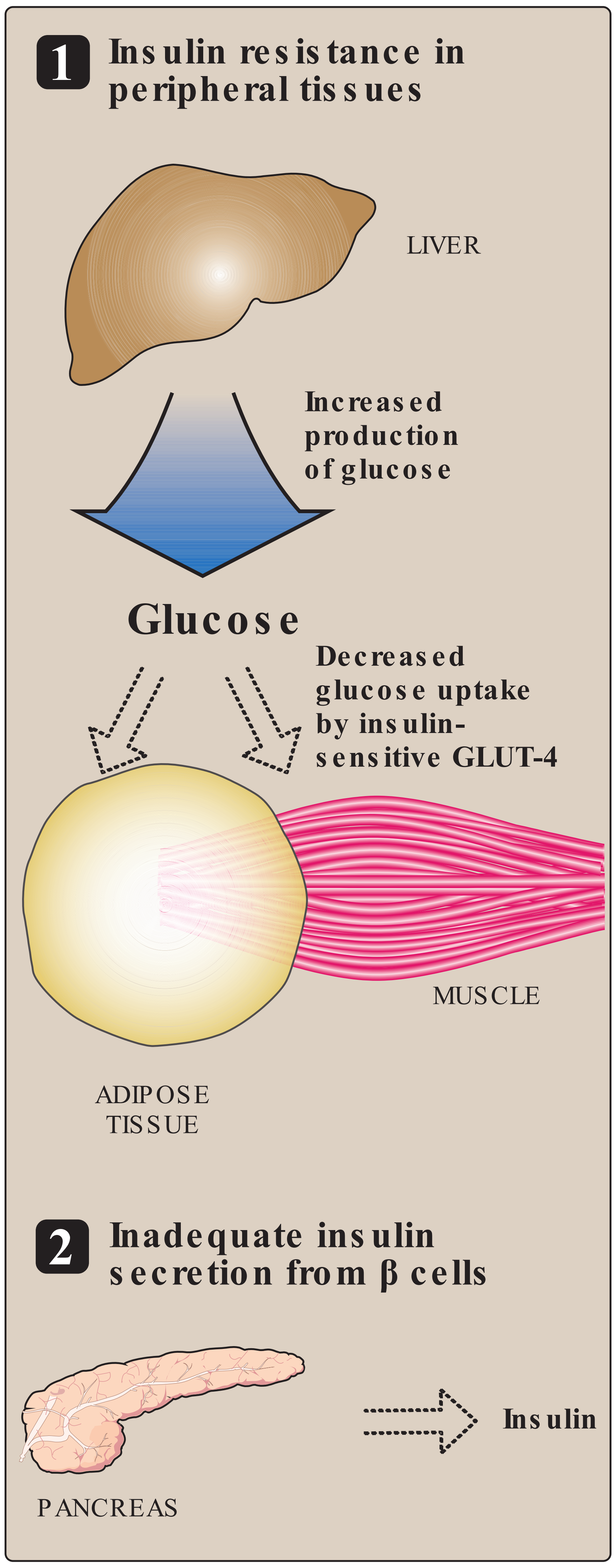

1 Insulin resistance in peripheral tissues
LIVER
Increased production of glucose
Glucose
Decreased glucose uptake by insulin-sensitive GLUT-4
MUSCLE
ADIPOSE TISSUE
2 Inadequate insulin secretion from β cells
Insulin
PANCREAS

Figure 25.6
Major factors contributing to hyperglycemia observed in type 2 diabetes. GLUT = glucose transporter.

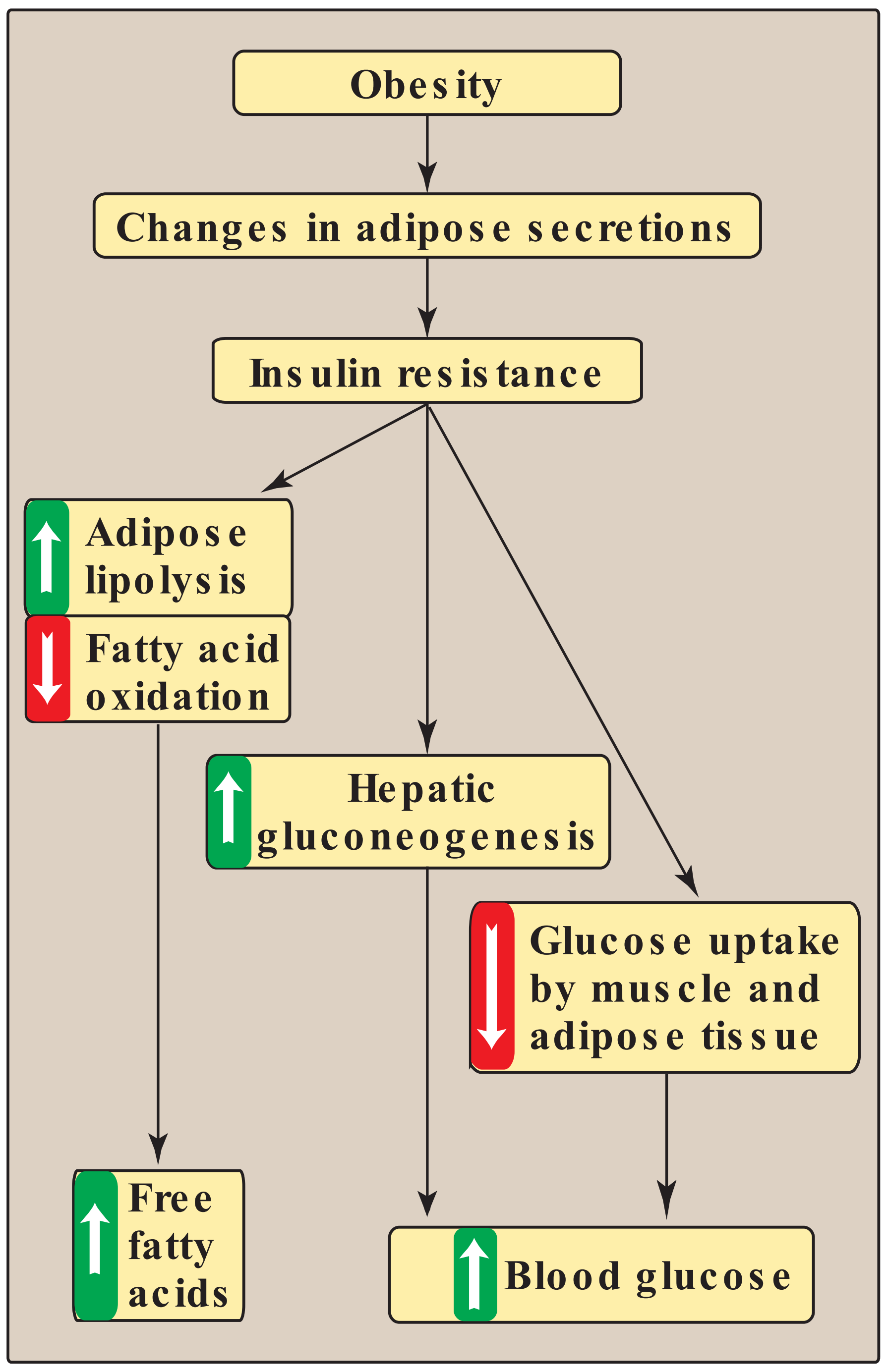

Obesity
Changes in adipose secretions
Insulin resistance
↑ Adipose lipolysis
↓ Fatty acid oxidation
↑ Hepatic gluconeogenesis
↓ Glucose uptake by muscle and adipose tissue
↑ Free fatty acids
↑ Blood glucose

Figure 25.7
Obesity, insulin resistance, and hyperglycemia. [Note: Inflammation also is associated with insulin resistance.]

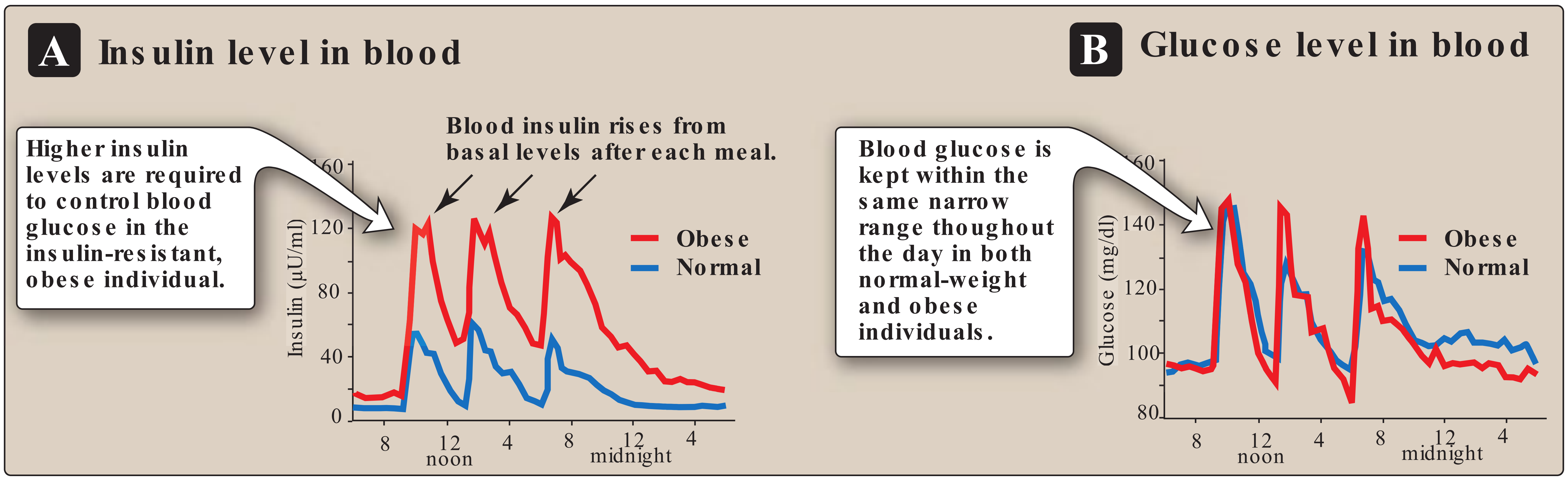
A. Insulin resistance

Insulin resistance is the decreased ability of target tissues, such as liver, adipose, and muscle, to respond properly to normal (or elevated) circulating concentrations of insulin. For example, insulin resistance is characterized by increased hepatic glucose production, decreased glucose uptake by muscle and adipose tissue, and increased adipose lipolysis with production of free fatty acids ([FFAs] Figure 25.7).

1. **Insulin resistance and obesity:** Although obesity is the most common cause of insulin resistance and T2D, most people with obesity and insulin resistance do not become diabetic. In the absence of a defect in β-cell function, nondiabetic, obese individuals can compensate for insulin resistance with elevated levels of insulin. For example, Figure 25.8A shows that insulin secretion is two to three times higher in obese subjects than it is in lean individuals. This higher insulin concentration compensates for the diminished effect of the hormone (as a result of insulin resistance) and produces blood glucose levels similar to those observed in lean individuals (Figure 25.8B).

2. **Insulin resistance and type 2 diabetes:** Insulin resistance alone will not lead to T2D. Rather, T2D develops in insulin-resistant individuals who also show impaired β-cell function. Insulin resistance and subsequent risk for the development of T2D is commonly observed in individuals who are obese, physically inactive, or elderly and in the 3%–5% of pregnant women who develop gestational diabetes. These patients are unable to sufficiently compensate for insulin resistance with increased insulin release. Figure 25.9 shows the time course for the development of hyperglycemia and the loss of β-cell function.

3. **Causes of insulin resistance:** Insulin resistance increases with weight gain and decreases with weight loss, and excess adipose tissue is key in the development of insulin resistance (see Figure 25.7). Adipose tissue is not simply an energy storage organ, but also a secretory organ. With obesity, there are changes in adipose secretions that result in insulin resistance. These include secretion


A Insulin level in blood

Higher insulin levels are required to control blood glucose in the insulin-resistant, obese individual.

Blood insulin rises from basal levels after each meal.

B Glucose level in blood

Blood glucose is kept within the same narrow range thoughout the day in both normal-weight and obese individuals.

Figure 25.8
Blood insulin and glucose levels in normal-weight and obese subjects.

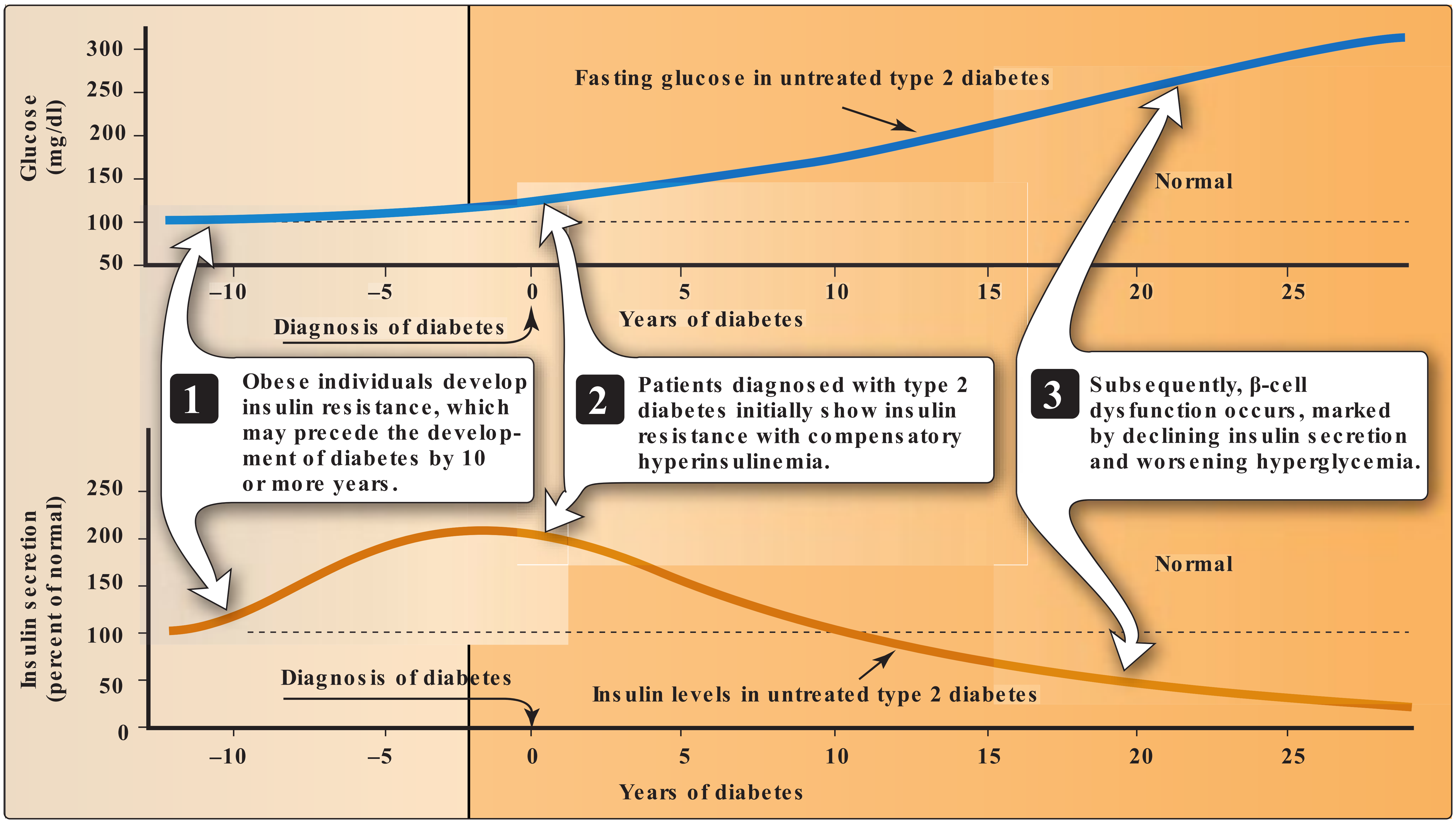


Figure 25.9
Progression of blood glucose and insulin levels in patients with type 2 diabetes.

of proinflammatory cytokines such as interleukin 6 (inflammation is associated with insulin resistance); increased synthesis of leptin, a protein with proinflammatory effects (see p. 353 for additional effects of leptin); and decreased secretion of adiponectin, an adipocyte protein with anti-inflammatory effects. One effect of insulin resistance is increased lipolysis and production of FFAs. FFA availability decreases use of glucose, contributing to hyperglycemia, and increases deposition of TAG in liver (hepatic steatosis). FFAs also have a proinflammatory effect. In the long-term, FFAs suppress glucose-induced insulin release. [Note: Adiponectin increases FA β-oxidation (see p. 353). Consequently, a decrease in this adipocyte protein contributes to FFA availability.]

B. Dysfunctional β cells

In T2D, the pancreas initially retains β-cell capacity, resulting in insulin levels that vary from above normal to below normal. However, with time, the β cell becomes increasingly dysfunctional and fails to secrete enough insulin to correct the prevailing hyperglycemia. For example, insulin levels are high in typical, obese, T2D patients but not as high as in similarly obese individuals who are nondiabetic. Thus, the natural progression of the disease results in a declining ability to control hyperglycemia with endogenous secretion of insulin (Figure 25.10). Deterioration of β-cell function may be accelerated by the toxic effects of sustained hyperglycemia and elevated FFAs and a proinflammatory environment.

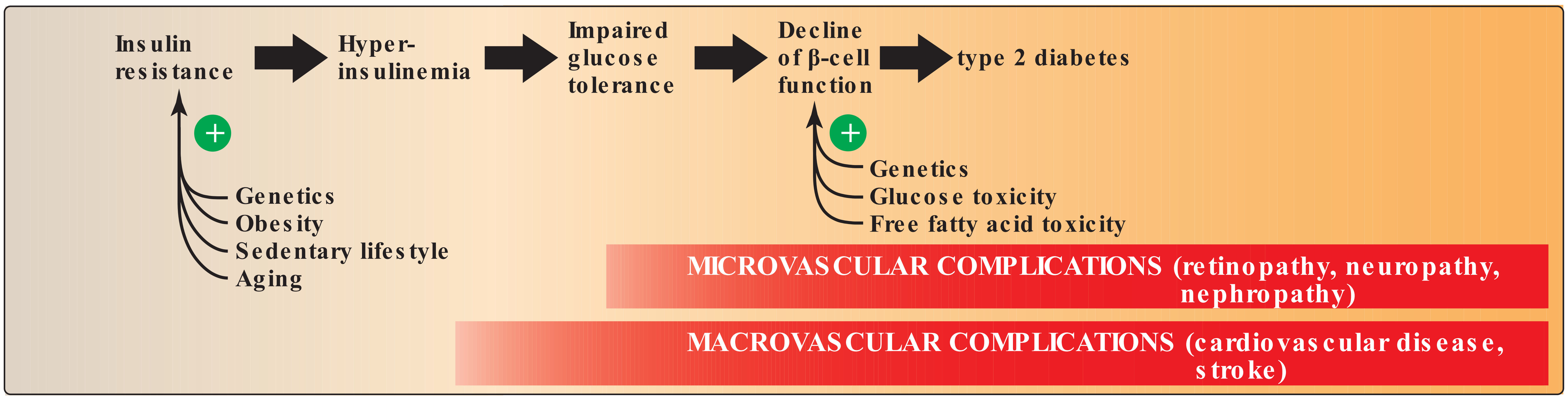

Insulin resistance
Hyper-insulinemia
Impaired glucose tolerance
Decline of β-cell function
type 2 diabetes
+
Genetics
Obesity
Sedentary lifestyle
Aging
+
Genetics
Glucose toxicity
Free fatty acid toxicity
MICROVASCULAR COMPLICATIONS (retinopathy, neuropathy, nephropathy)
MACROVASCULAR COMPLICATIONS (cardiovascular disease, stroke)

Figure 25.10
Typical progression of type 2 diabetes.

C. Metabolic changes in type 2 diabetes

The metabolic abnormalities of T2D are the result of insulin resistance expressed primarily in liver, muscle, and adipose tissue (Figure 25.11).

1. **Hyperglycemia:** Hyperglycemia is caused by increased hepatic production of glucose, combined with diminished peripheral use. Ketosis is usually minimal or absent in patients with T2D because the presence of insulin, even in the presence of insulin resistance, restrains hepatic ketogenesis.

2. **Dyslipidemia:** In the liver, FAs are converted to TAGs, which are packaged and secreted in VLDL. Chylomicrons are synthesized from dietary lipids by the intestinal mucosal cells following a meal (see p. 177). Because lipoprotein degradation catalyzed by lipoprotein lipase in adipose tissue is low in diabetics, the plasma chylomicron and VLDL levels are elevated, resulting in hypertriacylglycerolemia (see Figure 25.10). Low levels of high-density lipoproteins are also associated with T2D, likely as a result of increased degradation.

D. Treatment of type 2 diabetes

The goal in treating T2D is to maintain blood glucose concentrations within normal limits and to prevent the development of long-term complications. Weight reduction, exercise, and medical nutrition therapy (dietary modifications) often correct the hyperglycemia of newly diagnosed T2D. Hypoglycemic agents (for example, metformin, which decreases hepatic output of glucose), sulfonylureas (increase insulin secretion; see p. 310), thiazolidinediones (increase peripheral insulin sensitivity), α-glucosidase inhibitors (decrease absorption of dietary carbohydrate) or insulin therapy may be required to achieve satisfactory plasma glucose levels. [Note: Bariatric surgery in morbidly obese individuals with T2D has been shown to result in disease remission in most patients. Remission may not be permanent.]

IV. CHRONIC EFFECTS AND PREVENTION OF DIABETES

As noted previously, available therapies moderate the hyperglycemia of diabetes but fail to completely normalize metabolism. The long-standing elevation of blood glucose is associated with the chronic complications

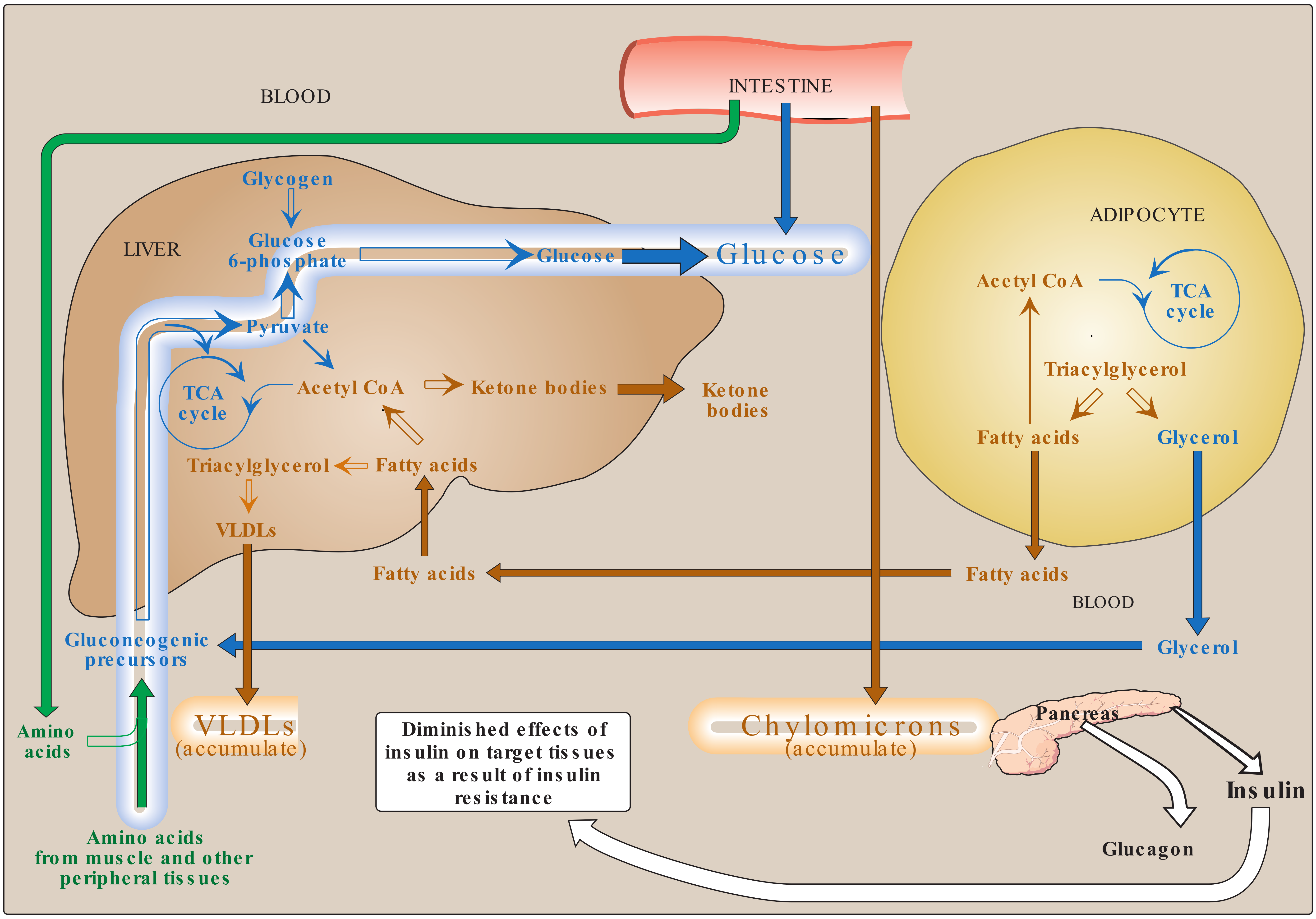

BLOOD
INTESTINE
Glycogen
LIVER
Glucose 6-phosphate
Glucose
Glucose
Pyruvate
TCA cycle
Acetyl CoA
Ketone bodies
Ketone bodies
Triacylglycerol
Fatty acids
VLDLs
Fatty acids
ADIPOCYTE
Acetyl CoA
TCA cycle
Triacylglycerol
Fatty acids
Glycerol
Fatty acids
BLOOD
Gluconeogenic precursors
Glycerol
Amino acids
VLDLs (accumulate)
Diminished effects of insulin on target tissues as a result of insulin resistance
Chylomicrons (accumulate)
Pancreas
Insulin
Glucagon
Amino acids from muscle and other peripheral tissues

Figure 25.11
Intertissue relationships in type 2 diabetes. [Note: Ketogenesis is restrained as long as insulin action is adequate.] TCA = tricarboxylic acid; CoA = coenzyme A; VLDL = very-low-density lipoprotein.

of diabetes including premature atherosclerosis as well as cardiovascular disease and stroke, retinopathy, nephropathy, and neuropathy. Intensive treatment with insulin (see p. 340) delays the onset and slows the progression of these long-term complications. For example, the incidence of retinopathy decreases as control of blood glucose improves and HbA_{1c} levels decrease (Figure 25.12). The benefits of tight control of blood glucose outweigh the increased risk of severe hypoglycemia in most patients. How hyperglycemia causes the chronic complications of diabetes is unclear. In cells in which entry of glucose is not dependent on insulin, elevated blood glucose leads to increased intracellular glucose and its metabolites. For example, increased intracellular sorbitol contributes to the formation of cataracts (see p. 140) in diabetics. Additionally, hyperglycemia promotes the nonenzymic condensation of glucose with cellular proteins in a reaction analogous to the formation of HbA_{1c} (see p. 34). These glycated proteins undergo additional reactions and become advanced glycation end products (AGEs) that mediate some of the early microvascular changes of diabetes and can reduce wound healing. AGEs bind to their receptors (RAGEs), causing the release of proinflammatory molecules. There is currently no preventative treatment for T1D. The risk for T2D can be significantly decreased by a combined regimen of medical nutrition therapy, weight loss, exercise, and aggressive control of hypertension and dyslipidemias. For example, Figure 25.12 shows the incidence of disease in normal and overweight individuals with varying degrees of exercise. The beneficial effect of intensive therapy on cardiovascular disease has not been shown in individuals with long-standing T2D. In contrast, initial intensive control in individuals with newly diagnosed diabetes has long-term benefit in decreasing the risk of myocardial infarction, diabetes-related death, and overall death. The clinical evidence, thus, supports initiating intensive therapy with the goal of lowering HbA_{1c} levels to below 7% as early as possible in the course of diabetes.

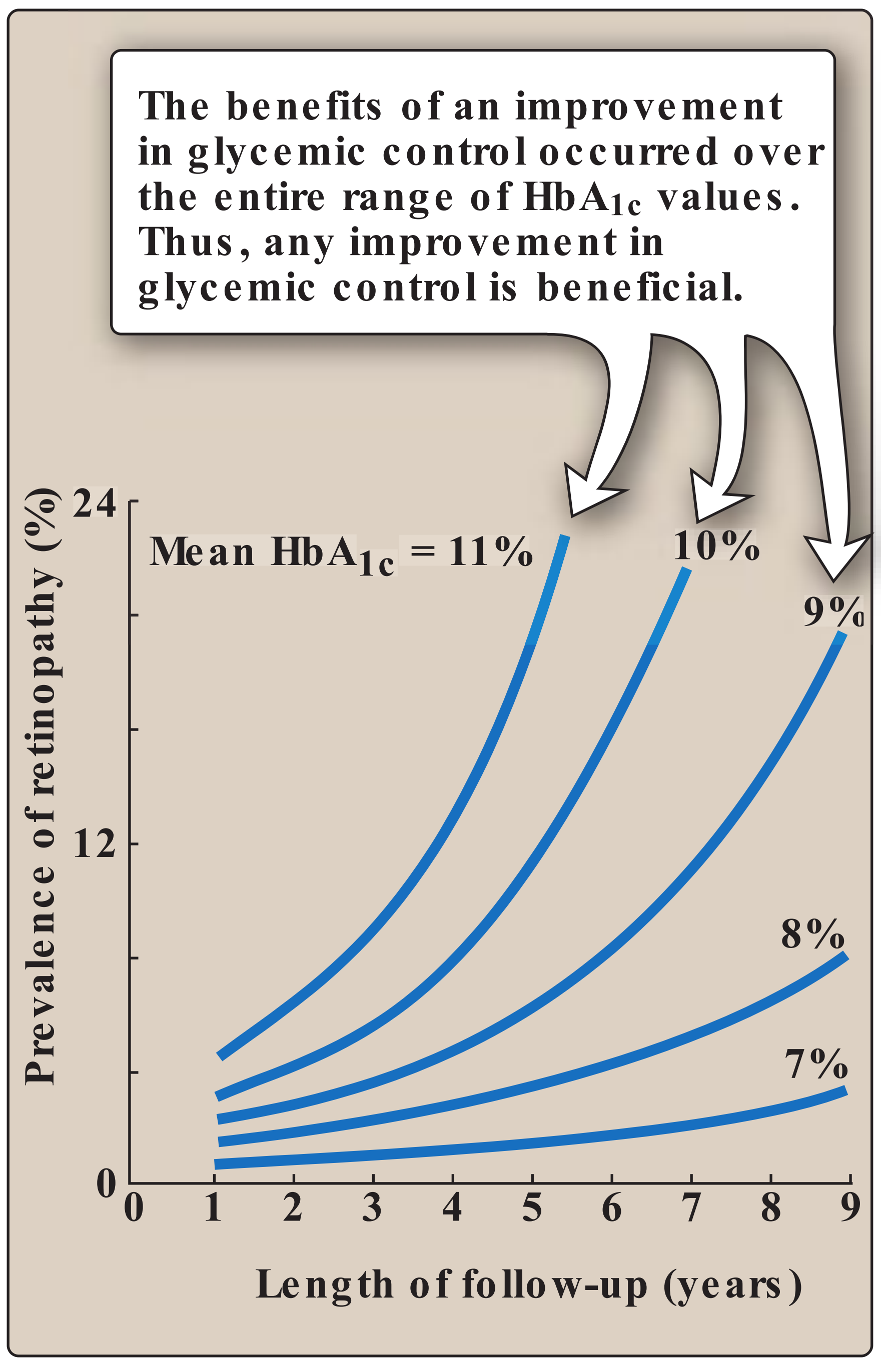
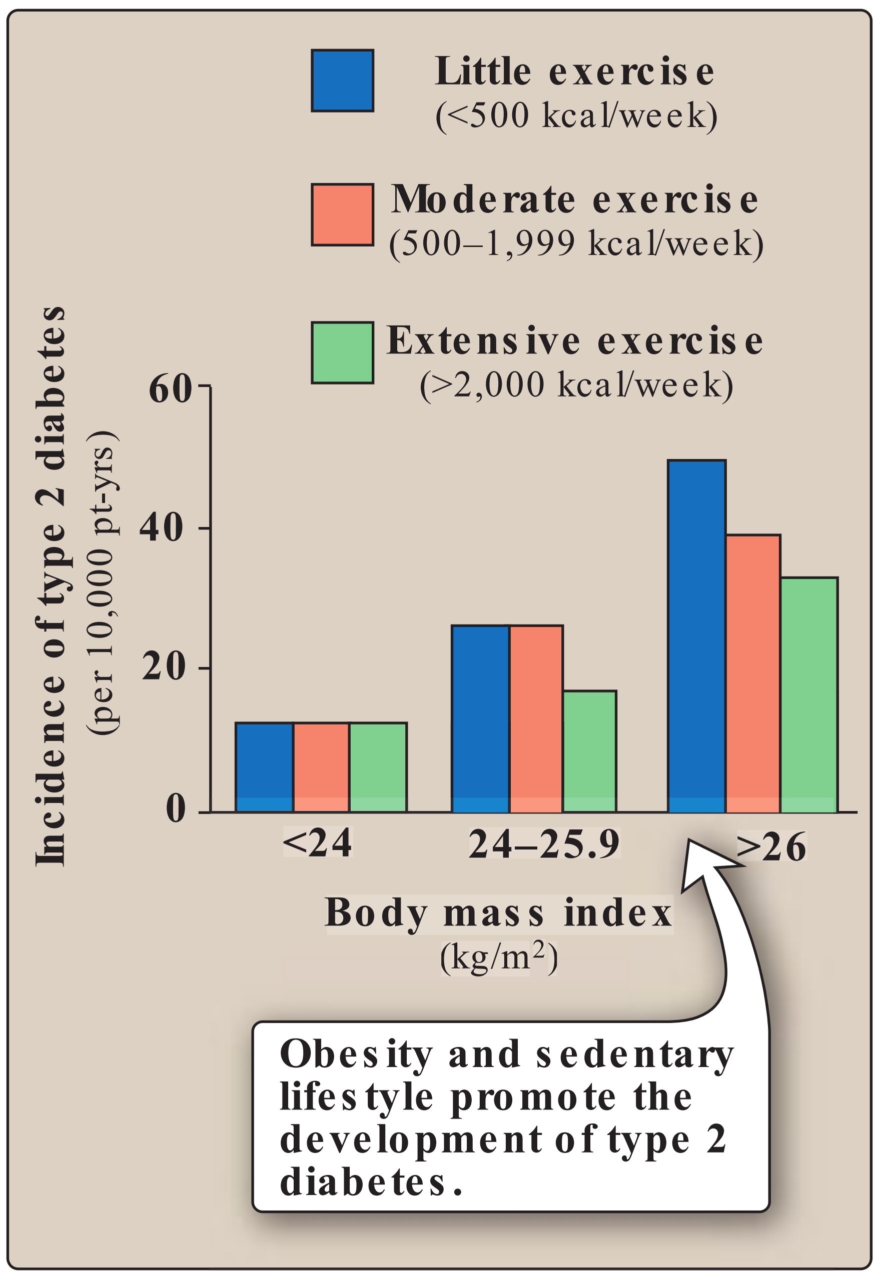

The benefits of an improvement in glycemic control occurred over the entire range of HbA_{1c} values. Thus, any improvement in glycemic control is beneficial.

Figure 25.12
Relationship of glycemic control and diabetic retinopathy. Hb = hemoglobin.



Obesity and sedentary lifestyle promote the development of type 2 diabetes.

Figure 25.13
Effect of body weight and exercise on the development of type 2 diabetes.

V. CHAPTER SUMMARY

Diabetes mellitus is a heterogeneous group of syndromes characterized by an **elevation of fasting blood glucose** that is caused by a relative or absolute deficiency of insulin (Figure 25.14). Diabetes is the leading cause of **adult blindness** and **amputation** and a major cause of **renal failure**, **nerve damage**, **heart attacks**, and **stroke**. Diabetes can be classified into two groups, **type 1** (T1D) and **type 2** (T2D). **Type 1** diabetics constitute approximately 10% of the nearly 26 million diabetics in the United States. The disease is characterized by an **absolute deficiency of insulin** caused by an **autoimmune attack** on the **β cells of the pancreas**. This destruction requires a **stimulus from the environment** (such as a viral infection) and a **genetic determinant** that allows the β cell to be recognized as "nonself." The **metabolic abnormalities** of T1D mellitus include **hyperglycemia**, **ketoacidosis**, and **hypertriacylglycerolemia** that result from a deficiency of insulin. Type 1 diabetics must rely on **exogenous insulin** delivered subcutaneously to control hyperglycemia and ketoacidosis. **T2D** has a strong **genetic** component. It results from a combination of **insulin resistance** and **dysfunctional β cells**. Insulin resistance is the decreased ability of target tissues, such as liver, adipose, and muscle, to respond properly to normal (or elevated) circulating concentrations of insulin. **Obesity** is the most common cause of insulin resistance. However, most people with obesity and insulin resistance do not become diabetic. In the absence of a defect in β-cell function, **nondiabetic**, **obese individuals** can compensate for insulin resistance with **elevated levels of insulin**. Insulin resistance alone will not lead to T2D. Rather, T2D develops in insulin-resistant individuals who also show impaired β-cell function. The **metabolic alterations** observed in T2D are **milder** than those described for the insulin-dependent form of the disease, in part, because insulin secretion in T2D, although inadequate, does restrain ketogenesis and blunts the development of diabetic ketoacidosis. Available treatments for diabetes moderate the hyperglycemia but fail to completely normalize metabolism. The long-standing elevation of blood glucose is associated with the **chronic complications of diabetes** including **premature atherosclerosis** (**macrovascular**) as well as **retinopathy**, **nephropathy**, and **neuropathy** (**microvascular**).

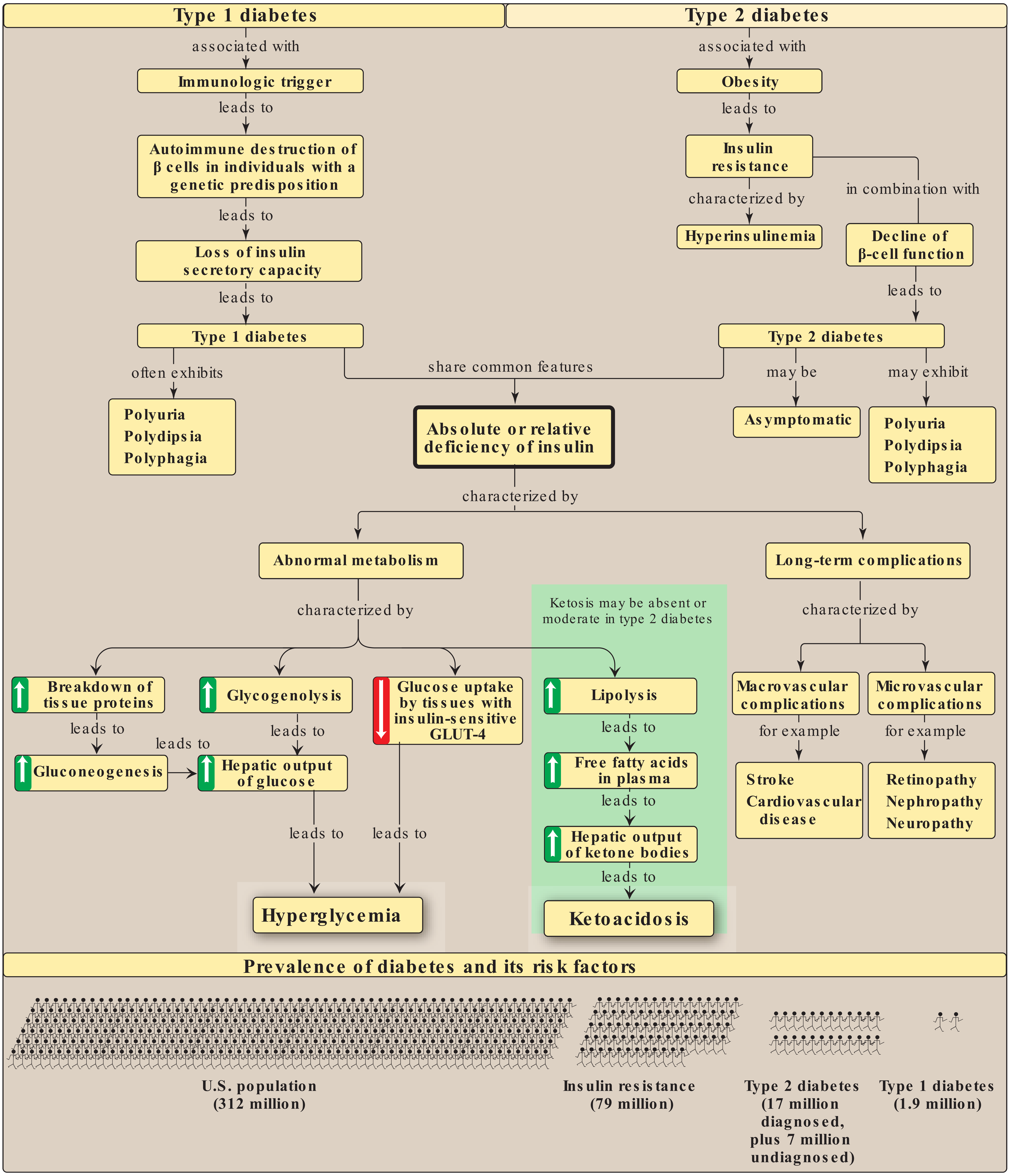

Type 1 diabetes
Type 2 diabetes
associated with
Immunologic trigger
leads to
Autoimmune destruction of β cells in individuals with a genetic predisposition
leads to
Loss of insulin secretory capacity
leads to
Type 1 diabetes
often exhibits
Polyuria
Polydipsia
Polyphagia
associated with
Obesity
leads to
Insulin resistance
characterized by
Hyperinsulinemia
in combination with
Decline of β-cell function
leads to
Type 2 diabetes
may be
Asymptomatic
may exhibit
Polyuria
Polydipsia
Polyphagia
share common features
Absolute or relative deficiency of insulin
characterized by
Abnormal metabolism
characterized by
Breakdown of tissue proteins
leads to
Gluconeogenesis
leads to
Glycogenolysis
leads to
Hepatic output of glucose
Glucose uptake by tissues with insulin-sensitive GLUT-4
leads to
leads to
Hyperglycemia
Ketosis may be absent or moderate in type 2 diabetes
Lipolysis
leads to
Free fatty acids in plasma
leads to
Hepatic output of ketone bodies
leads to
Ketoacidosis
Long-term complications
characterized by
Macrovascular complications
for example
Stroke
Cardiovascular disease
Microvascular complications
for example
Retinopathy
Nephropathy
Neuropathy
Prevalence of diabetes and its risk factors
U.S. population (312 million)
Insulin resistance (79 million)
Type 2 diabetes (17 million diagnosed, plus 7 million undiagnosed)
Type 1 diabetes (1.9 million)

Figure 25.14
Key concept map for diabetes. GLUT = glucose transporter.

Obesity

26

I. OVERVIEW

Obesity is a disorder of body weight regulatory systems characterized by an accumulation of excess body fat. In primitive societies, in which daily life required a high level of physical activity and food was only available intermittently, a genetic tendency favoring storage of excess calories as fat may have had a survival value. Today, however, the sedentary lifestyle and abundance and wide variety of palatable, inexpensive foods in industrialized societies has undoubtedly contributed to an obesity epidemic. As adiposity has increased, so has the risk of developing associated diseases, such as type 2 diabetes (T2D), cardiovascular disease, hypertension, cancer, and arthritis. Particularly alarming is the explosion of obesity in children and adolescents, which has shown a threefold increase in prevalence over the last two decades. [Note: Approximately 17% of those ages 2-19 years are obese.] In the United States, the lifetime risk of becoming overweight or obese is approximately 50% and 25%, respectively. Obesity has increased globally. In fact, by some estimates, there are more obese than undernourished individuals worldwide.

II. ASSESSMENT OF OBESITY

Because the amount of body fat is difficult to measure directly, it is usually determined from an indirect measure, the body mass index (BMI), which has been shown to correlate with the amount of body fat in most individuals. [Note: Exceptions are athletes who have large amounts of lean muscle mass.] Measuring the waist size with a tape measure is also used to screen for obesity, because this measurement reflects the amount of fat in the central abdominal area of the body. The presence of excess central fat is associated with an increased risk for morbidity and mortality, independent of the BMI. [Note: A waist size ≥ 40 inches in men and ≥ 35 inches in women is considered a risk factor.]

A. Body mass index

The BMI (weight in kg)/(height in meters)^2 provides a measure of relative weight, adjusted for height. This allows comparisons both within and between populations. The healthy range for the BMI is between 18.5 and 24.9. Individuals with a BMI between 25 and 29.9 are considered overweight, those with a BMI equal to or greater than 30 are defined as obese, and a BMI over 40 is considered extremely obese. Anyone more than 100 pounds overweight is considered severely (morbidly) obese (Figure 26.1). These cutoffs are based on the studies examining the relationship of BMI to premature death and are similar in men and women. Nearly two thirds of American adults are overweight, and more than one third of those are obese.

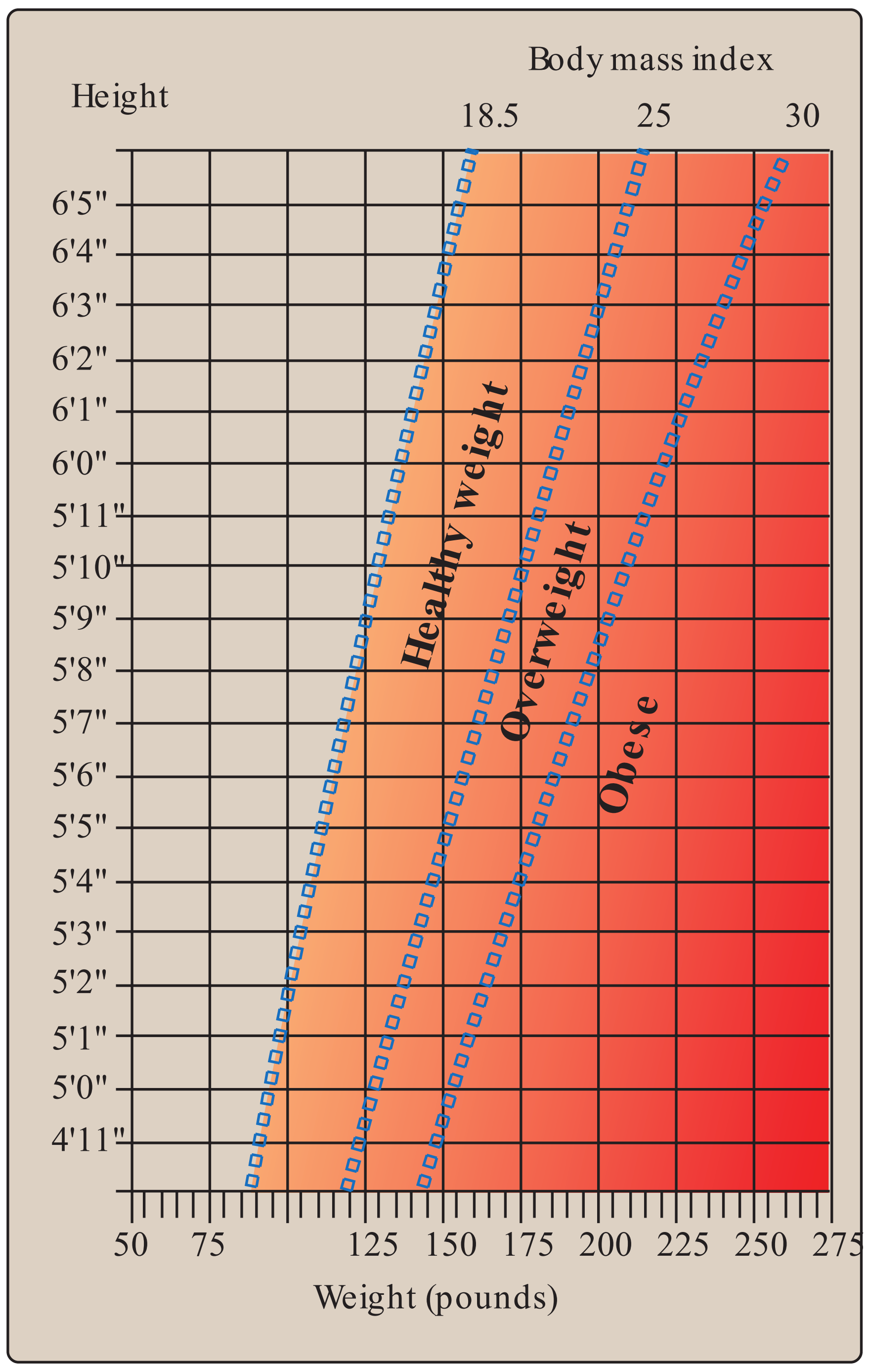


Figure 26.1
Body mass index (BMI) Chart. To use the BMI Chart, find height in the left-hand column. Move across the row to weight. Height and weight intersect at the individual's BMI. [Note: To calculate BMI using inches and pounds, use BMI = [weight in pounds/(height in inches)^2] × 703.

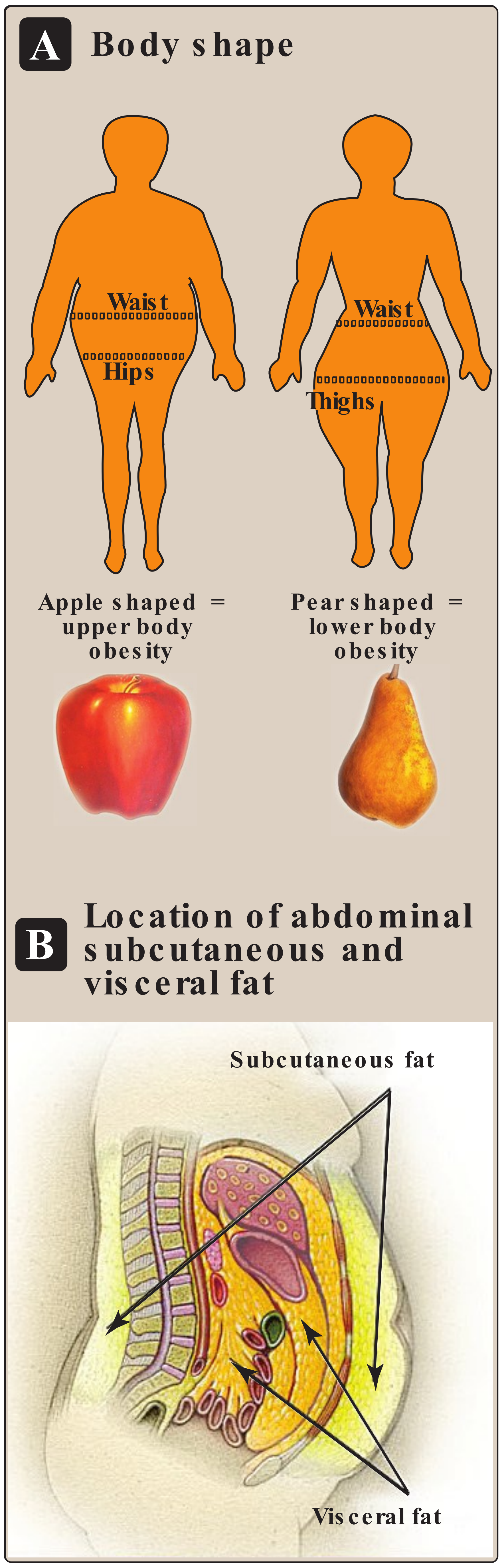

A
Body shape
Waist
Hips
Waist
Thighs
Apple shaped = upper body obesity
Pear shaped = lower body obesity
B
Location of abdominal subcutaneous and visceral fat
Subcutaneous fat
Visceral fat

Figure 26.2
A. Individuals with upper body obesity (left) have greater health risks than individuals with lower body obesity (right). B. Visceral fat is located inside the abdominal cavity, packed in between the internal organs. Subcutaneous fat is found underneath the skin.

B. Anatomic differences in fat deposition

The anatomic distribution of body fat has a major influence on associated health risks. A waist-to-hip ratio of more than 0.8 for women and more than 1.0 for men is defined as android, "apple-shaped," or upper body obesity, and is associated with more fat deposition in the trunk (Figure 26.2A). In contrast, a lower waist/hip ratio reflects a preponderance of fat distributed in the hips and thighs and is called gynoid, "pear-shaped," or lower body obesity. It is defined as a waist/hip ratio of less than 0.8 for women and less than 1.0 for men. The pear shape, more commonly found in women, presents a much lower risk of metabolic disease, and some studies indicate that it may actually be protective. Thus, the clinician can use simple indices of body shape to identify those who may be at higher risk for metabolic diseases associated with obesity.

About 80%–90% of the fat stored in the human body is in subcutaneous depots, just under the skin, in the abdominal (upper body) and the gluteal-femoral (lower body) regions. In addition, 10%–20% of body fat is stored in so-called visceral depots (omental and mesenteric), which are located within the abdominal cavity in close association with the digestive tract (Figure 26.2B). Excess fat in visceral and abdominal subcutaneous stores increases health risks associated with obesity.

C. Biochemical differences in regional fat depots

The regional types of fat described above are biochemically different. Subcutaneous adipocytes from the lower body (gluteal-femoral), particularly in women, are larger, very efficient at fat (triacylglycerol [TAG]) deposition, and tend to mobilize fatty acids more slowly than those from the abdominal subcutaneous depots. Visceral adipocytes are the most metabolically active. Both abdominal subcutaneous and visceral depots of obese subjects have high rates of lipolysis and contribute to increased availability of free fatty acids (FFAs). These metabolic differences may contribute to the higher risk found in individuals with upper body obesity.

1. **Endocrine function:** White adipose tissue, once thought to be a passive reservoir of TAGs, is now known to play an active role in body weight regulatory systems. For example, the adipocyte is an endocrine cell that secretes a number of protein regulators, such as the hormones leptin and adiponectin. Leptin regulates appetite as well as metabolism (see p. 353). Adiponectin reduces levels of FFAs in the blood and has been associated with improved lipid profiles, increased insulin sensitivity resulting in better glycemic control, and reduced inflammation in diabetic patients. [Note: Adiponectin levels decrease as body weight increases, and leptin levels increase.]
2. **Importance of portal circulation:** With obesity, there is increased release of FFAs and secretion of proinflammatory cytokines, such as interleukin 6 (IL-6), from adipose tissue. [Note: Cytokines are small proteins that regulate the immune system.] One reason that visceral and abdominal adipose depots may have such a large influence on metabolic dysfunction in obesity is that the FFAs and cytokines released from these depots enter the portal vein and, therefore, have direct access to the liver. In the liver,

they may lead to insulin resistance (see p. 342) and increased hepatic synthesis of TAGs, which are released as components of very-low-density lipoprotein particles and contribute to the hypertriacylglycerolemia associated with obesity. By contrast, FFAs from lower body subcutaneous adipose depots enter the general circulation, where they can be oxidized in muscle and, therefore, reach the liver in lower concentration.

D. Size and number of fat cells

As TAGs are stored, adipocytes can expand to an average of two to three times their normal volume. (Figure 26.3). However, the ability of a fat cell to expand is limited. With prolonged overnutrition, preadipocytes within adipose tissue are stimulated to proliferate and differentiate into mature fat cells, increasing the number of adipocytes. Thus, most obesity is due to a combination of increased fat cell size (hypertrophy) and number (hyperplasia). Like other tissues, the adipose tissue undergoes continuous remodeling. Contrary to early dogma, we now know that adipocytes can die. The estimated average lifespan of an adipocyte is 10 years.

Obese individuals can have up to five times the normal number of fat cells. If excess calories cannot be accommodated within adipose tissue, the excess fatty acids "spill over" into other tissues, such as muscle and liver. The amount of this so-called "ectopic fat" is strongly associated with insulin resistance. With weight loss in an obese individual, the size of the fat cells is reduced, but the number of fat cells is not usually affected. Thus, a normal body fat is achieved by decreasing the size of the fat cell below normal. Small fat cells are very efficient at reaccumulating fat, and this may drive appetite and weight regain.

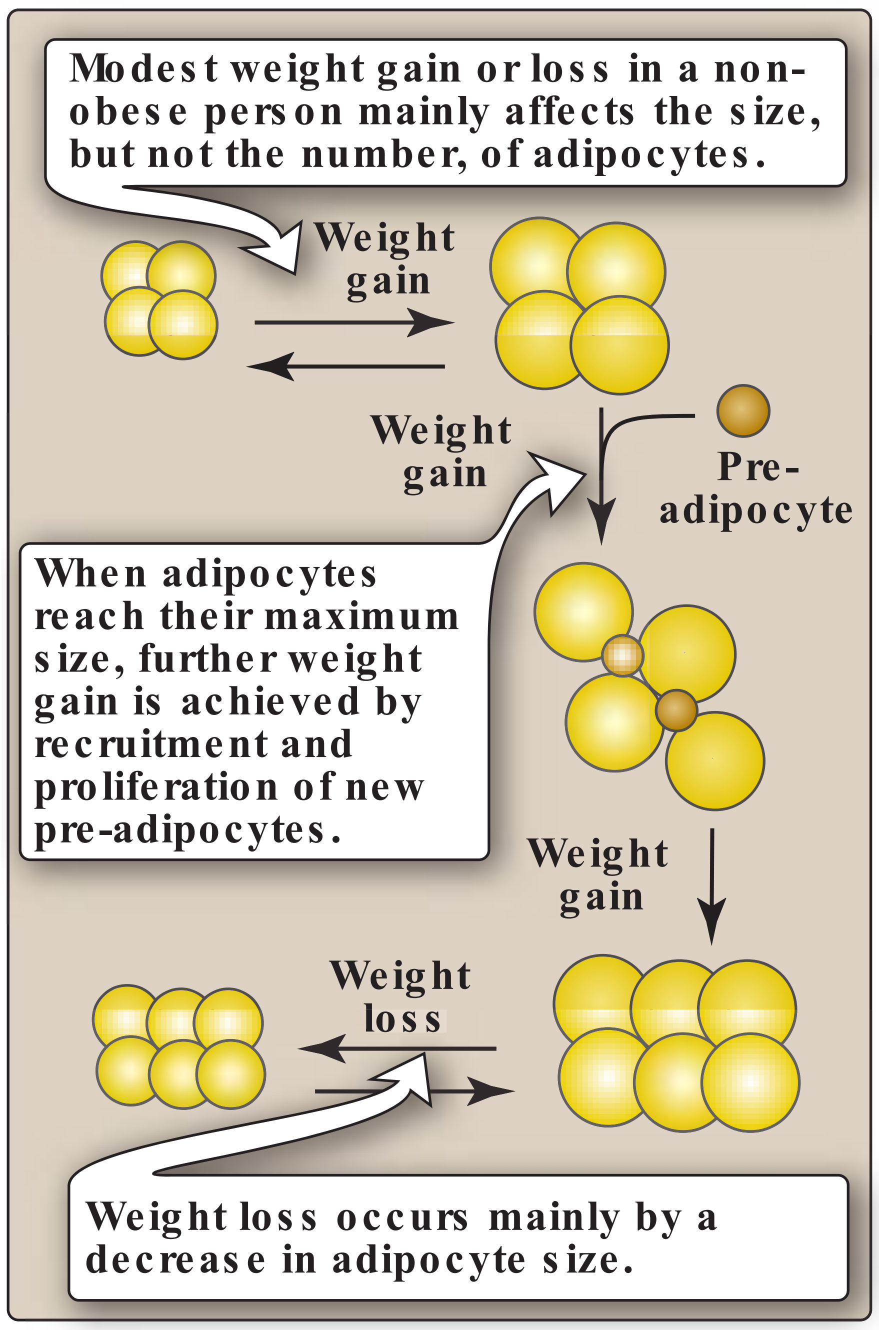

Modest weight gain or loss in a non-obese person mainly affects the size, but not the number, of adipocytes.
Weight gain
Weight gain
Pre-adipocyte
When adipocytes reach their maximum size, further weight gain is achieved by recruitment and proliferation of new pre-adipocytes.
Weight gain
Weight loss
Weight loss occurs mainly by a decrease in adipocyte size.

Figure 26.3
Hypertrophic (increased size) and hyperplastic (increased number) changes to adipocytes are thought to occur in severe obesity.

III. BODY WEIGHT REGULATION

The body weight of most individuals tends to be relatively stable over time. This observation prompted the hypothesis that each individual has a biologically predetermined "set point" for body weight. The body attempts to add to adipose stores when the body weight falls below the set point and to lose adipose from stores when the body weight is higher than the set point. Thus, the body defends the set point. For example, with weight loss, appetite increases and energy expenditure falls, whereas with overfeeding, appetite falls and energy expenditure may slightly increase (Figure 26.4). However, a strict set point model explains neither why some individuals fail to revert to their starting weight after a period of overeating nor the current epidemic of obesity.

A. Genetic contributions to obesity

It is now evident that genetic mechanisms play a major role in determining body weight.

1. **Biologic origin:** The importance of genetics as a determinant of obesity is indicated by the observation that children who are adopted usually show a body weight that correlates with their biologic rather than adoptive parents. Furthermore, identical twins have very similar BMIs (Figure 26.5), whether reared together or apart, and their BMIs are more similar than those of nonidentical, dizygotic twins.

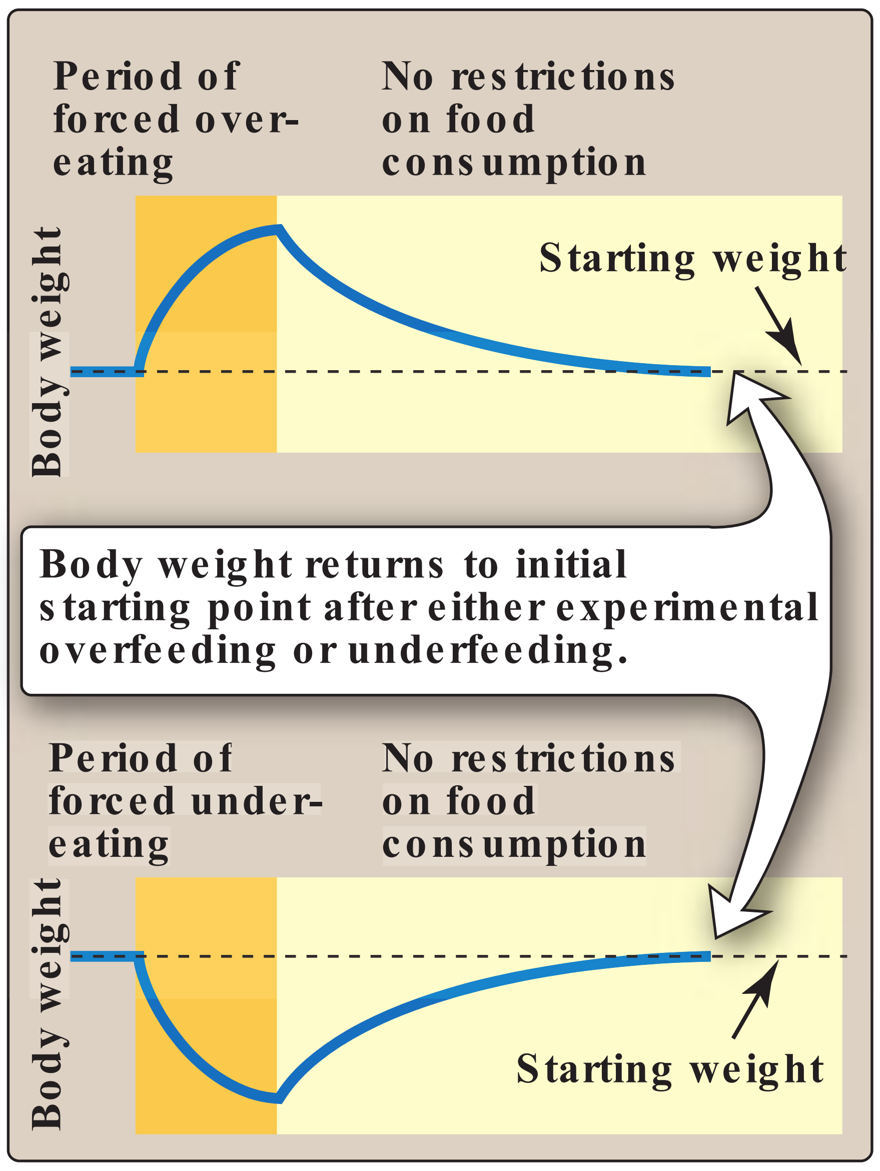


Figure 26.4
Weight changes following episodes of overfeeding or underfeeding followed by feeding with no restrictions.

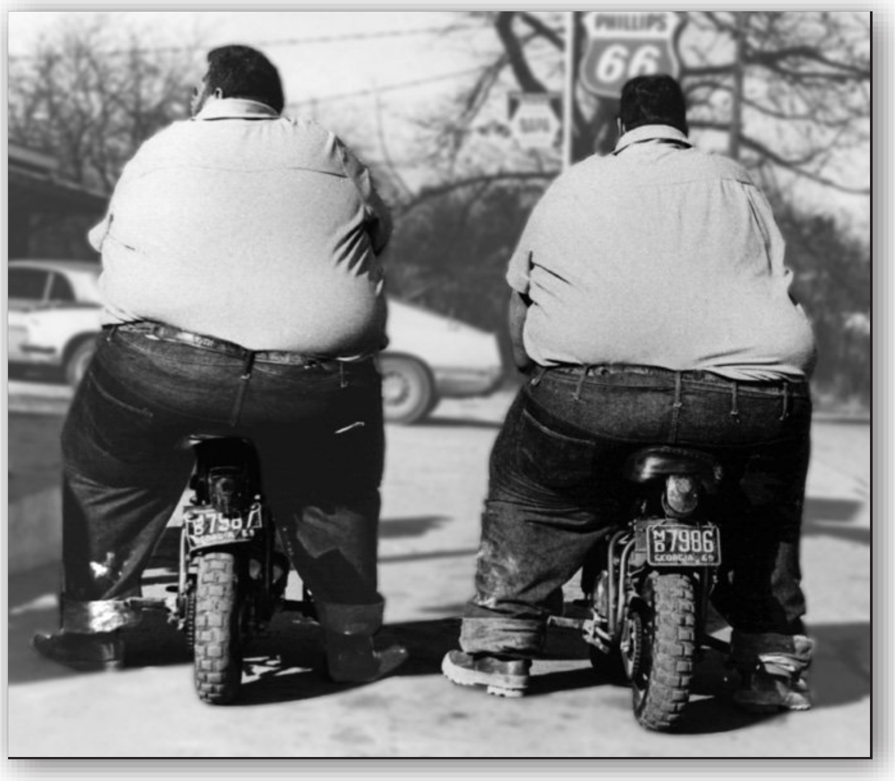


Figure 26.5
Identical twins with combined weight of 1,300 pounds. Note similarity in body shape.

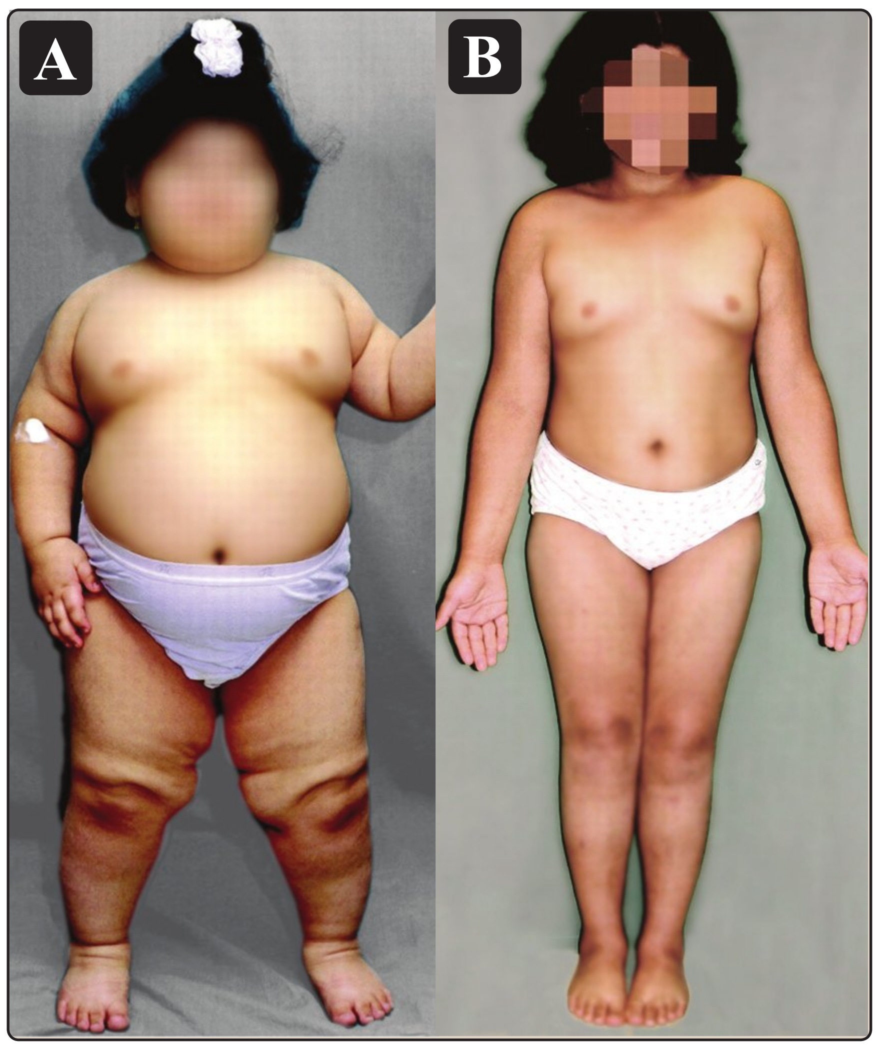


Figure 26.6
A. Patient with leptin deficiency before initiation of therapy at age 5 years. B. Patient at age 9 years after 48 months of therapy with subcutaneous injection of recombinant leptin.

2. **Mutations:** Rare, single gene mutations can cause human obesity. For example, mutations in the gene for the adipocyte hormone leptin or its receptor produce hyperphagia (increased appetite for and consumption of food) and massive obesity (Figure 26.6), underscoring the importance of the leptin system in regulating human body weight (Section IV). Most obese humans have elevated leptin levels but appear to be resistant to the appetite-regulating effects of this hormone.

B. Environmental and behavioral contributions

The epidemic of obesity occurring over the last decade cannot be simply explained by changes in genetic factors, which are stable on this short time scale. Clearly, environmental factors, such as the ready availability of palatable, energy-dense foods, play a role in the increased prevalence of obesity. Furthermore, sedentary lifestyles encouraged by TV watching, automobiles, computer usage, and energy-sparing devices in the workplace and at home decrease physical activity and enhance the tendency to gain weight. Eating behaviors, such as snacking, portion size, variety of foods consumed, an individual's unique food preferences, and the number of people present during eating also influence food consumption. It is important to note, however, that in this same environment, many individuals do not become obese. The susceptibility to obesity appears to be explained, at least in part, by an interaction of an individual's genes and his or her environment and can be influenced by additional factors such as maternal under- or overnutrition that may "set" the body regulatory systems to defend a higher or lower level of body fat. Epigenetic changes (see p. 409), therefore, likely influence the risk for obesity.

IV. MOLECULES THAT INFLUENCE OBESITY

The cause of obesity can be summarized in a deceptively simple statement of the first law of thermodynamics: Obesity results when energy (caloric) intake exceeds energy expenditure. However, the mechanism underlying this imbalance involves a complex interaction of biochemical, neurologic, environmental, and psychologic factors. The basic neural and humoral pathways that regulate appetite, energy expenditure, and body weight involve systems that regulate short-term food intake (meal to meal), and signals for the long-term (day to day, week to week, year to year) regulation of body weight (Figure 26.7).

A. Long-term signals

Long-term signals reflect the status of TAG stores.

1. **Leptin:** Leptin is an adipocyte peptide hormone that is secreted in proportion to the size of fat stores. When we consume fewer calories than we need, body fat declines, and leptin production from the fat cell decreases. The body adapts by minimizing energy utilization (decreasing activity) and increasing appetite. Unfortunately, in many individuals, the leptin system may be better at preventing weight loss than preventing weight gain. Although a meal or overeating increases leptin and this should,

in theory, also dampen appetite (an anorexigenic effect) and prevent overconsumption of calories, other cues that stimulate appetite can apparently overcome the leptin system in many individuals. [Note: Leptin's effects are mediated through binding to its receptors in the arcuate nucleus of the hypothalamus.]

2. **Insulin:** Obese individuals are also hyperinsulinemic. Like leptin, insulin acts on hypothalamic neurons to dampen appetite. (See Chapter 23 for the effects of insulin on metabolism.)

B. Short-term signals

Short-term signals from the gastrointestinal tract control hunger and satiety, which affect the size and number of meals over a time course of minutes to hours. In the absence of food intake (between meals), the stomach produces ghrelin, an orexigenic (appetite-stimulating) hormone that drives hunger. As food is consumed, gut hormones, including cholecystokinin (CCK) and peptide YY, among others, induce satiety (an anorexigenic effect), thereby terminating eating, through actions on gastric emptying and neural signals to the hypothalamus. Within the hypothalamus, neuropeptides such as neuropeptide Y (orexigenic) and α-melanocyte–stimulating hormone (α-MSH), which is anorexigenic, and neurotransmitters, such as serotonin and dopamine, are important in regulating hunger and satiety. Long-term and short-term signals interact, insofar as leptin can affect the sensitivity of hypothalamic neurons to short-term signals such as CCK. Thus, there are many and complex regulatory loops that control the size and number of meals in relationship to the status of body fat stores. [Note: α-MSH binds to the melanocortin-4 receptor (MC4R). Loss-of-function mutations to MC4R are associated with early-onset obesity.]

V. METABOLIC CHANGES IN OBESITY

The primary metabolic effects of obesity include dyslipidemias, glucose intolerance, and insulin resistance expressed primarily in the liver, muscle, and adipose tissue. These metabolic abnormalities reflect molecular signals originating from the increased mass of adipocytes. (see Figure 25.7 and Figure 26.7)

A. Metabolic syndrome

Abdominal obesity is associated with a cluster of metabolic abnormalities that is referred to as the metabolic syndrome and includes glucose intolerance (hyperglycemia below that classified as diabetes; see p. 338), insulin resistance, hyperinsulinemia, dyslipidemia (low levels of high-density lipoprotein and elevated TAGs), and hypertension (Figure 26.8). The metabolic syndrome is also associated with a state of low-grade, chronic, systemic inflammation that contributes to the pathogenesis of insulin resistance and atherosclerosis. In obesity, adipocytes release proinflammatory mediators such as IL-6. Additionally, low levels of the adipocyte hormone adiponectin that normally dampens inflammation and sensitizes tissues, especially the liver, to insulin, may contribute to the metabolic syndrome and, therefore, the risk of T2D and heart disease.

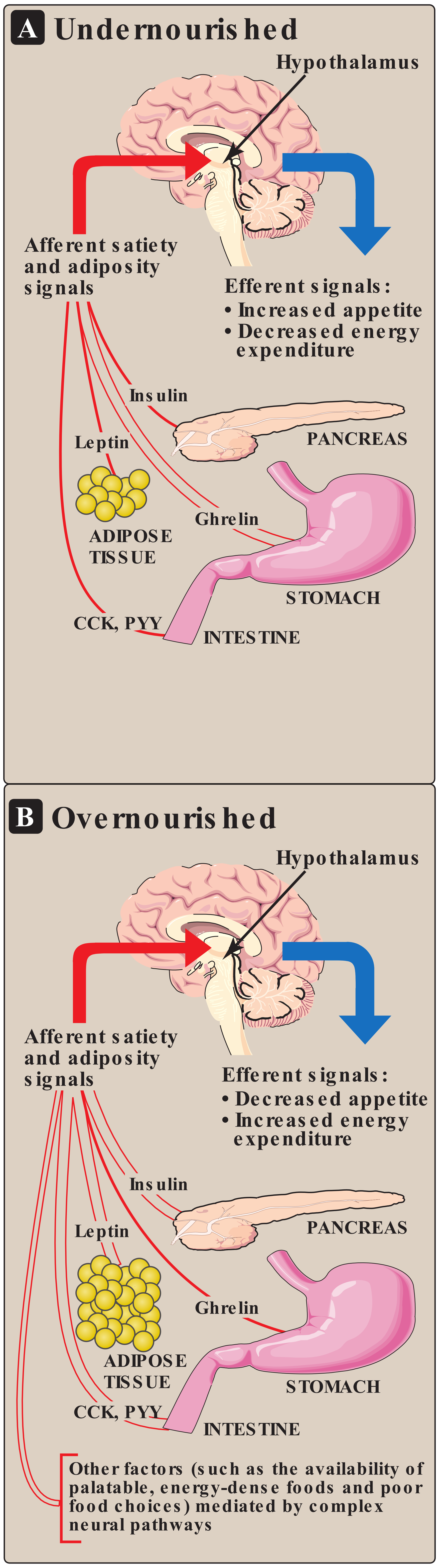

A Undernourished
Hypothalamus
Afferent satiety and adiposity signals
Efferent signals:
• Increased appetite
• Decreased energy expenditure
Insulin
PANCREAS
Leptin
ADIPOSE TISSUE
Ghrelin
STOMACH
CCK, PYY
INTESTINE
B Overnourished
Hypothalamus
Afferent satiety and adiposity signals
Efferent signals:
• Decreased appetite
• Increased energy expenditure
Insulin
PANCREAS
Leptin
ADIPOSE TISSUE
Ghrelin
STOMACH
CCK, PYY
INTESTINE
Other factors (such as the availability of palatable, energy-dense foods and poor food choices) mediated by complex neural pathways

Figure 26.7
Some signals that influence appetite and satiety. CCK = cholecystokinin, PYY = peptide YY.

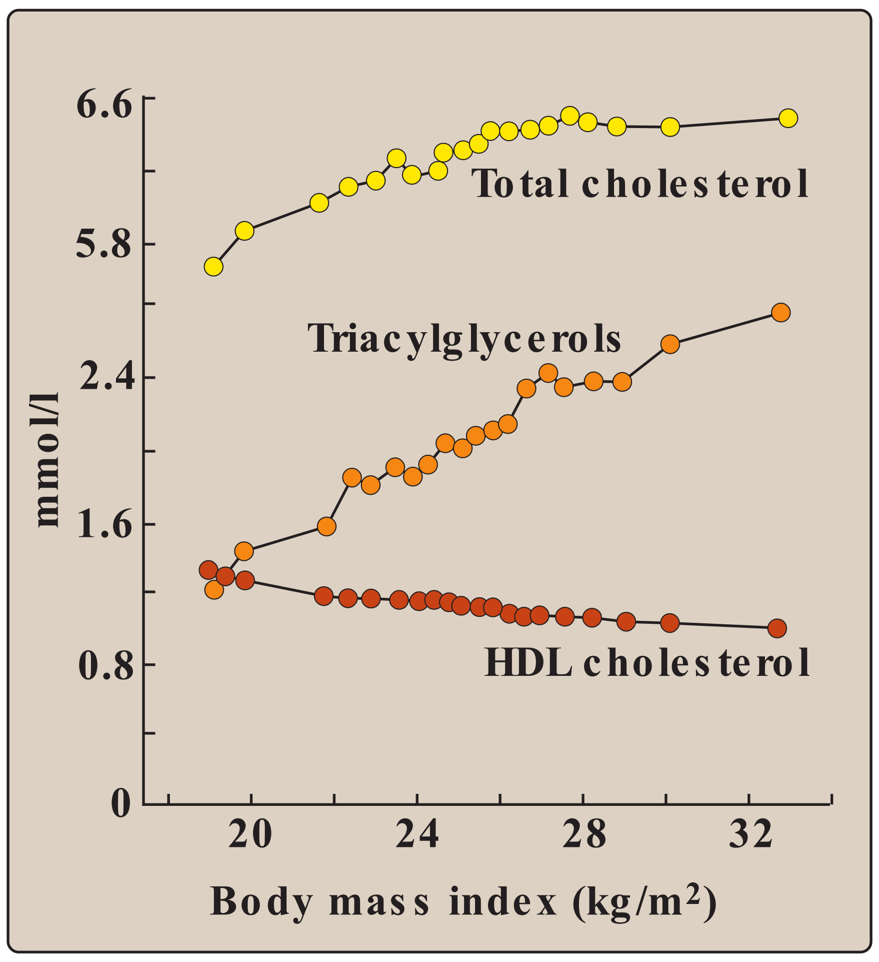


Figure 26.8
Body mass index and changes in blood lipids. HDL= high-density lipoprotein.

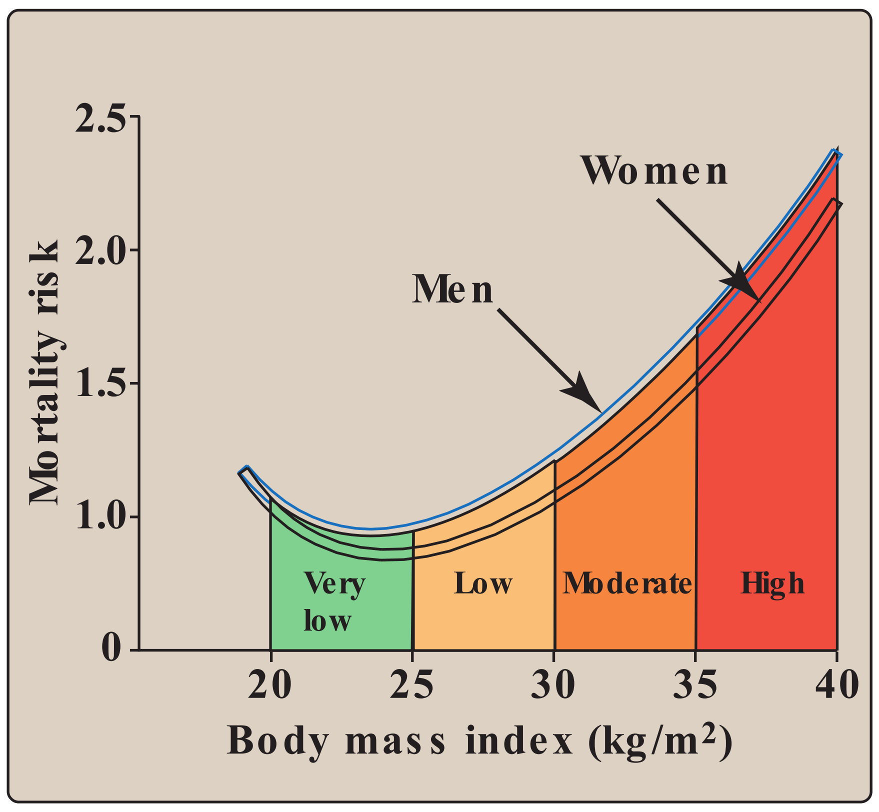


Figure 26.9
Body mass index and the relative risk of death.

VI. OBESITY AND HEALTH

Obesity is correlated with an increased risk of death (Figure 26.9) and is a risk factor for a number of chronic conditions, including T2D, dyslipidemias, hypertension, heart disease, some cancers, gallstones, arthritis, gout, pelvic floor disorders (for example, urinary incontinence), nonalcoholic fatty liver disease, and sleep apnea. The relationship between obesity and associated morbidities is stronger among individuals younger than age 55 years. After age 74 years, there is no longer an association between increased BMI and mortality. Weight loss in obese individuals leads to decreased blood pressure, serum TAGs, and blood glucose levels. High-density lipoproteins increase.

VII. WEIGHT REDUCTION

Weight reduction can help reduce the complications of obesity, including T2D and hypertension. To achieve weight reduction, the obese patient must decrease energy intake or increase energy expenditure, although decreasing energy intake is thought to contribute more to inducing weight loss. Typically, a prescription for weight reduction combines dietary change; increased physical activity; and behavioral modification, which can include nutritional education and meal planning, recording and monitoring food intake through food diaries, modifying factors that lead to overeating, and relearning cues to satiety. Once weight loss is achieved, weight maintenance is a separate process that requires vigilance because the majority of patients regain weight after they stop their weight loss efforts.

A. Physical activity

An increase in physical activity can create an energy deficit. Although adding exercise to a hypocaloric regimen may not produce a greater weight loss initially, exercise is a key component of programs directed at maintaining weight loss. In addition, physical activity increases cardiopulmonary fitness and reduces the risk of cardiovascular disease, independent of weight loss. Persons who combine caloric restriction and exercise with behavioral treatment may expect to lose about 5%–10% of initial body weight over a period of 4–6 months. Studies show that individuals who maintain their exercise program regain less weight after their initial weight loss.

B. Caloric restriction

Dieting is the most commonly practiced approach to weight control. Because 1 pound of adipose tissue corresponds to approximately 3,500 kcal, one can estimate the effect that caloric restriction will have on the amount of adipose tissue. Weight loss on calorie-restricted diets is determined primarily by energy intake and not nutrient composition. [Note: Compositional aspects can, however, affect glycemic control and the blood lipid profile.] Caloric restriction is ineffective over the long term for many individuals. More than 90% of people who attempt to lose weight regain the lost weight when dietary intervention is suspended. Nonetheless, it is important to recognize that, although few individuals will reach their ideal weight with treatment, weight losses of 10% of body weight over a 6-month period often reduce blood pressure and lipid levels and enhance control of T2D. The health benefits of even relatively small weight losses should, therefore, be emphasized to the patient.

C. Pharmacologic treatment

Several weight-loss medications are currently approved by the U.S. Food and Drug Administration for use in adults with a BMI of 30 or higher. Three approved for long-term use are: 1) orlistat (decreases absorption of dietary fat), 2) lorcaserin (promotes satiety), and 3) a combination of phentermine (suppresses appetite) and extended-release topiramate (controls seizures). [Note: Phentermine monotherapy is approved for short-term use only.] Their effects on weight reduction tend to be modest, and weight regain upon termination of drug therapy is common.

D. Surgical treatment

Gastric bypass and restriction surgeries are effective in causing weight loss in severely obese individuals. Through mechanisms that remain poorly understood, these operations greatly improve poor blood sugar control in morbidly obese diabetic individuals.

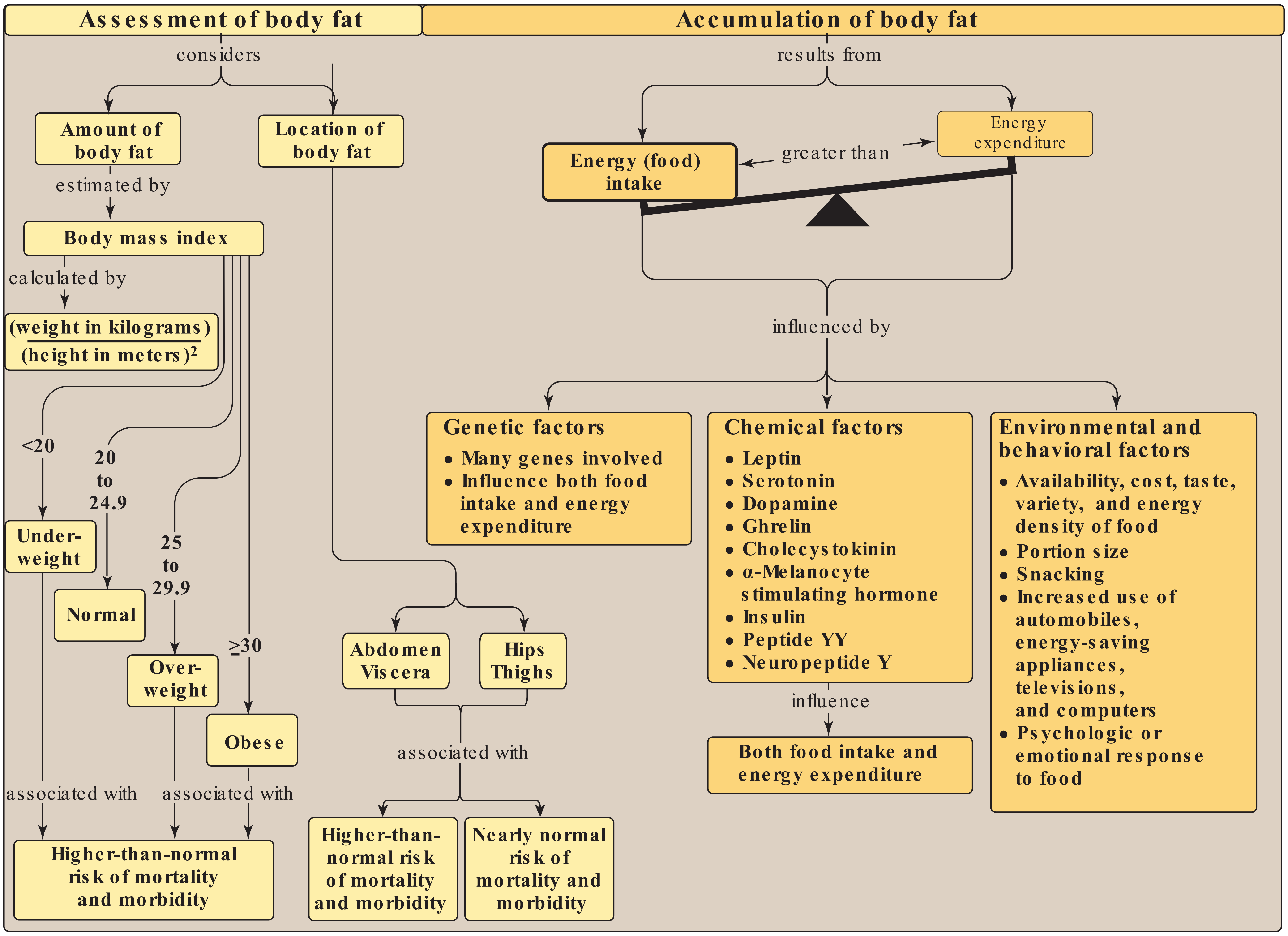

Assessment of body fat
considers
Amount of body fat
Location of body fat
estimated by
Body mass index
calculated by
(weight in kilograms) / (height in meters)^2
<20
20 to 24.9
25 to 29.9
≥30
Underweight
Normal
Overweight
Obese
associated with
associated with
Higher-than-normal risk of mortality and morbidity
Abdomen Viscera
Hips Thighs
associated with
Higher-than-normal risk of mortality and morbidity
Nearly normal risk of mortality and morbidity
Accumulation of body fat
results from
Energy (food) intake
greater than
Energy expenditure
influenced by
Genetic factors
• Many genes involved
• Influence both food intake and energy expenditure
Chemical factors
• Leptin
• Serotonin
• Dopamine
• Ghrelin
• Cholecystokinin
• α-Melanocyte stimulating hormone
• Insulin
• Peptide YY
• Neuropeptide Y
influence
Both food intake and energy expenditure
Environmental and behavioral factors
• Availability, cost, taste, variety, and energy density of food
• Portion size
• Snacking
• Increased use of automobiles, energy-saving appliances, televisions, and computers
• Psychologic or emotional response to food

Figure 26.10
Key concept map for obesity.

VIII. CHAPTER SUMMARY

Obesity, the accumulation of excess body fat, results when energy (caloric) intake exceeds energy expenditure. Obesity is increasing in industrialized countries because of a reduction in daily energy expenditure and an increase in energy intake resulting from the increasing availability of palatable, inexpensive foods. The **body mass index** (**BMI**) is easy to determine and highly correlated to body fat. Nearly two thirds of U.S. adults are **overweight** (BMI $\geq$ 25 kg/m^2) and more than one third of this group are **obese** (BMI $\geq$ 30 kg/m^2). The anatomic distribution of body fat has a major influence on associated health risks. Excess fat located in the **central abdominal** area is associated with greater risk for hypertension, insulin resistance, diabetes, dyslipidemia, and coronary heart disease as compared to fat located in the hips and thighs. A person's weight is determined by genetic and environmental factors. **Appetite** is influenced by **afferent**, or incoming, **signals** (that is, neural signals, circulating hormones, and metabolites) that are integrated by the **hypothalamus**. These diverse signals prompt release of hypothalamic peptides and activate outgoing, efferent neural signals. **Obesity** is correlated with an **increased risk of death** and is also a risk factor for a number of chronic conditions. **Weight reduction** is achieved best with negative energy balance, that is, by **decreasing caloric intake**. Virtually all diets that limit particular groups of foods or macronutrients lead to short-term weight loss. Long-term maintenance of weight loss is difficult to achieve. Modest reduction in food intake occurs with **pharmacologic treatment. Surgical procedures**, such as gastric bypass, designed to limit food intake are an option for the severely obese patient who has not responded to other treatments.

Nutrition

27

I. OVERVIEW

Nutrients are the constituents of food necessary to sustain the normal functions of the body. All energy is provided by three classes of nutrients: fats, carbohydrates, and protein, with ethanol providing calories in some diets (Figure 27.1). Because the intake of these energy-rich molecules is larger than that of the other dietary nutrients, they are called macronutrients. This chapter focuses on the kinds and amounts of macronutrients that are needed to maintain optimal health and prevent chronic disease in adults. Those nutrients needed in lesser amounts, vitamins and minerals, are called micronutrients and are considered in Chapter 28.

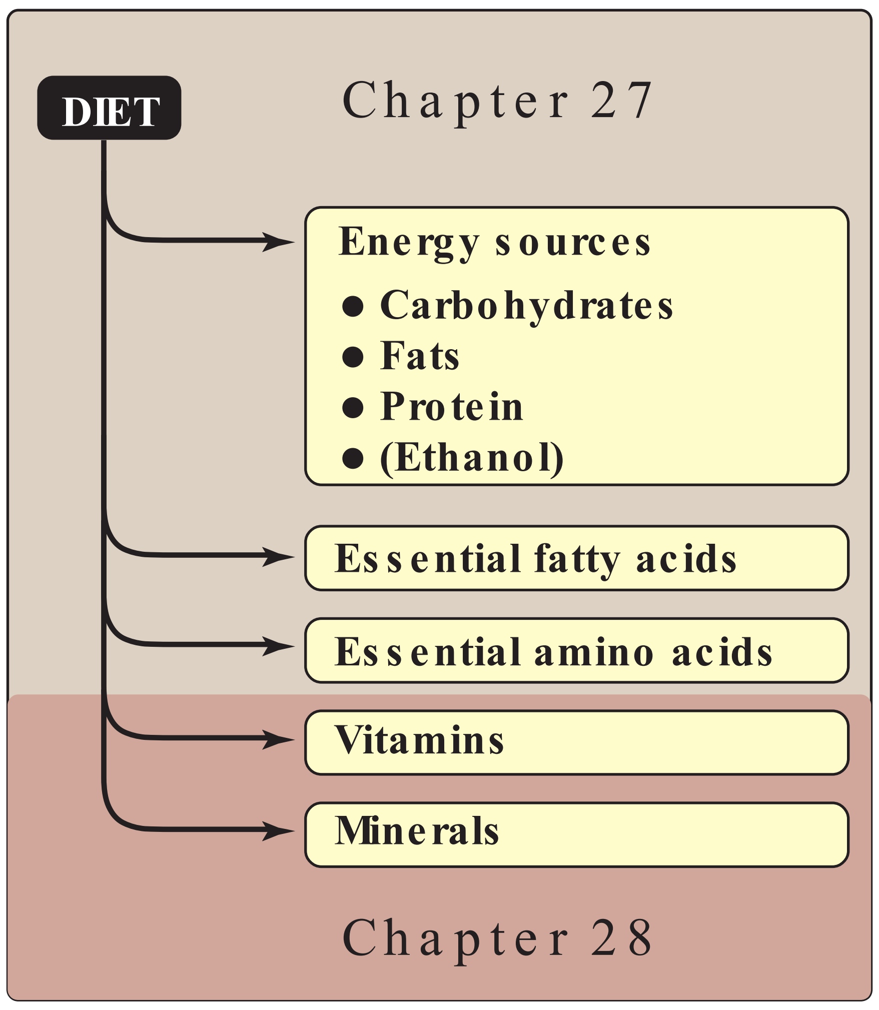


Figure 27.1
Essential nutrients obtained from the diet. [Note: Ethanol is not considered an essential component of the diet but may provide a significant contribution to the daily caloric intake of some individuals.]

II. DIETARY REFERENCE INTAKES

Committees of U.S. and Canadian experts organized by the Food and Nutrition Board of the Institute of Medicine of the National Academy of Sciences have compiled Dietary Reference Intakes (DRIs), which are estimates of the amounts of nutrients required to prevent deficiencies and maintain optimal health and growth. The DRI expands on the Recommended Dietary Allowances (RDAs), which have been published with periodic revisions since 1941. Unlike the RDA, the DRI establishes upper limits on the consumption of some nutrients and incorporates the role of nutrients in lifelong health, going beyond deficiency diseases. Both the DRI and the RDA refer to long-term average daily nutrient intakes, because it is not necessary to consume the full RDA every day.

A. Definition of Dietary Reference Intake

The DRIs consist of four dietary reference standards for the intake of nutrients designated for specific life stage (age) groups, physiologic states, and gender (Figure 27.2).

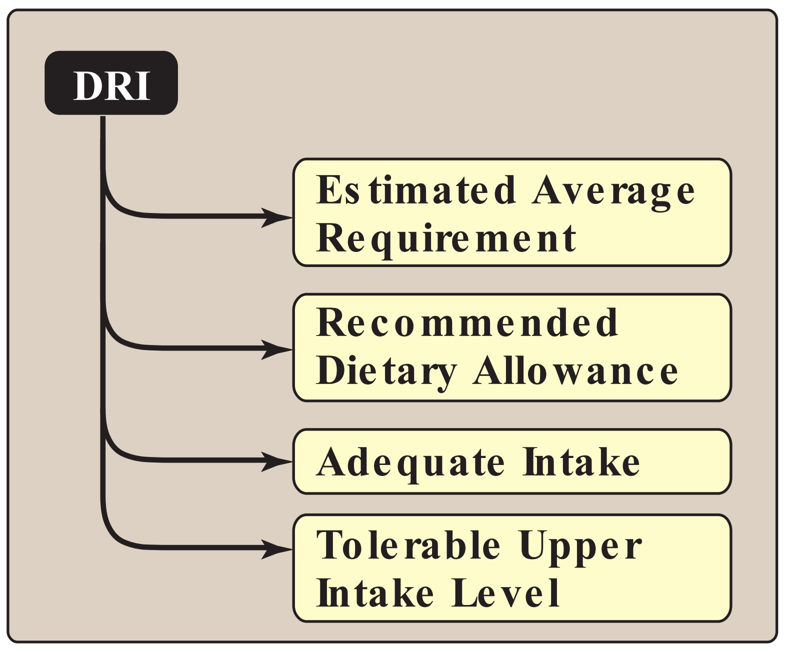

DRI
Estimated Average Requirement
Recommended Dietary Allowance
Adequate Intake
Tolerable Upper Intake Level

Figure 27.2
Components of the Dietary Reference Intakes (DRI).

1. **Estimated Average Requirement:** The average daily nutrient intake level estimated to meet the requirement of one half of the healthy individuals in a particular life stage and gender group is the Estimated Average Requirement (EAR). It is useful in estimating the actual requirements in groups and individuals.

2. **Recommended Dietary Allowance:** The RDA is the average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97%–98%) individuals in a life stage and gender group. The RDA is not the minimal requirement for healthy individuals, but it is intentionally set to provide a margin of safety for most individuals. The EAR serves as the foundation for setting the RDA. If the standard deviation (SD) of the EAR is available and the requirement for the nutrient is normally distributed, the RDA is set at 2 SDs above the EAR (that is, RDA = EAR + $2SD_{EAR}$).

NUTRIENT	EAR, RDA, or AI	UL
Thiamine	EAR, RDA	—
Riboflavin	EAR, RDA	—
Niacin	EAR, RDA	UL
Vitamin B_6	EAR, RDA	UL
Folate	EAR, RDA	UL
Vitamin B_{12}	EAR, RDA	—
Pantothenic acid	AI	—
Biotin	AI	—
Choline	AI	UL
Vitamin C	EAR, RDA	UL
Vitamin A	EAR, RDA	UL
Vitamin D	EAR, RDA	UL
Vitamin E	EAR, RDA	UL
Vitamin K	AI	—
Boron	—	UL
Calcium	EAR, RDA	UL
Chromium	AI	—
Copper	EAR, RDA	UL
Fluoride	AI	UL
Iodine	EAR, RDA	UL
Iron	EAR, RDA	UL
Magnesium	EAR, RDA	UL
Manganese	AI	UL
Molybdenum	EAR, RDA	UL
Nickel	—	UL
Phosphorus	EAR, RDA	UL
Selenium	EAR, RDA	UL
Vanadium	—	UL
Zinc	EAR, RDA	UL

Figure 27.3
Dietary Reference Intakes for vitamins and minerals in individuals age 1 year and older. EAR = Estimated Average Requirement; RDA = Recommended Dietary Allowance; AI = Adequate Intake; UL = Tolerable Upper Intake Level; — = no value established.

3. **Adequate Intake:** An Adequate Intake (AI) is set instead of an RDA if sufficient scientific evidence is not available to calculate an EAR or RDA. The AI is based on estimates of nutrient intake by a group (or groups) of apparently healthy people. For example, the AI for young infants, for whom human milk is the recommended sole source of food for the first 4–6 months, is based on the estimated daily mean nutrient intake supplied by human milk for healthy, full-term infants who are exclusively breastfed.

4. **Tolerable Upper Intake Level:** The highest average daily nutrient intake level that is likely to pose no risk of adverse health effects to almost all individuals in the general population is the Tolerable Upper Intake Level (UL). As intake increases above the UL, the potential risk of adverse effects may increase. The UL is useful because of the increased availability of fortified foods and the increased use of dietary supplements. For some nutrients, there may be insufficient data on which to develop a UL.

B. Using the Dietary Reference Intakes

Most nutrients have a set of DRIs (Figure 27.3). Usually a nutrient has an EAR and a corresponding RDA. Most are set by age and gender and may be influenced by special factors, such as pregnancy and lactation in women. When the data are not sufficient to estimate an EAR (or an RDA), an AI is designated. Intakes below the EAR need to be improved because the probability of adequacy is 50% or less (Figure 27.4). Intakes between the EAR and RDA likely need to be improved because the probability of adequacy is less than 98%, and intakes at or above the RDA can be considered adequate. Intakes above the AI can be considered adequate. Intakes between the UL and the RDA can be considered to have no risk for adverse effects. [Note: The DRI is designed to meet the nutritional needs of the healthy and, thus, does not include any special needs of the sick.]

III. ENERGY REQUIREMENT IN HUMANS

The Estimated Energy Requirement (EER) is the average dietary energy intake predicted to maintain an energy balance (that is, when the calories consumed are equal to the energy expended) in a healthy adult of a defined age, gender, and height whose weight and level of physical activity are consistent with good health. Differences in the genetics, body composition, metabolism, and behavior of individuals make it difficult to accurately predict a person's caloric requirements. However, some simple approximations can provide useful estimates. For example, sedentary adults require about 30 kcal/kg/day to maintain body weight, moderately active adults require 35 kcal/kg/day, and very active adults require 40 kcal/kg/day. [Note: The EER that is listed on food labels is either 2,000 or 2,500 kcal/day.]

A. Energy content of food

The energy content of food is calculated from the heat released by the total combustion of food in a calorimeter. It is expressed in kilocalories (kcal, or Cal). The standard conversion factors for determining the metabolic caloric value of fat, protein, and carbohydrate are shown in Figure 27.5. Note that the energy content of fat is more than twice that of carbohydrate or protein, whereas the energy

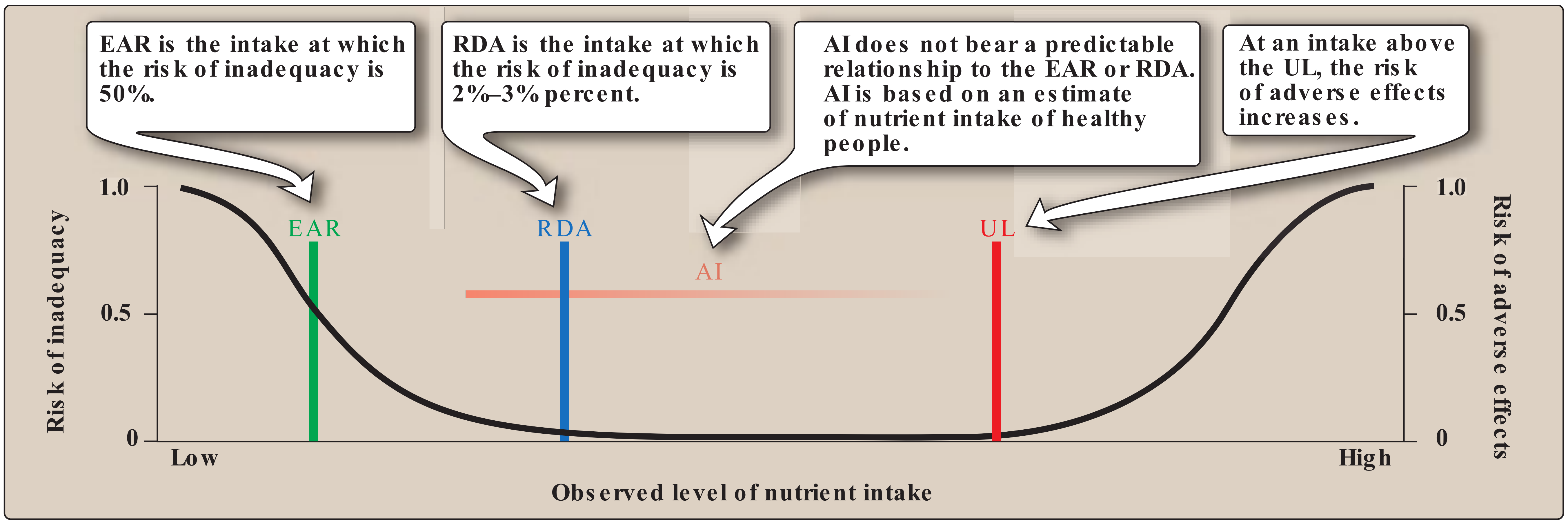

EAR is the intake at which the risk of inadequacy is 50%.
RDA is the intake at which the risk of inadequacy is 2%–3% percent.
AI does not bear a predictable relationship to the EAR or RDA. AI is based on an estimate of nutrient intake of healthy people.
At an intake above the UL, the risk of adverse effects increases.
Risk of inadequacy
1.0
0.5
0
EAR
RDA
AI
UL
Low
High
Observed level of nutrient intake
Risk of adverse effects

Figure 27.4

Comparison of the components of the Dietary Reference Intakes. EAR = Estimated Average Requirement; RDA = Recommended Dietary Allowance; AI = Adequate Intake; UL = Tolerable Upper Intake Level.

content of ethanol is intermediate between those of fat and carbohydrate. [Note: The joule (J) is a unit of energy widely used in countries other than the United States. One cal = 4.2 J; 1 Cal (1 kcal, 1 food calorie) = 4.2 kJ. For uniformity, many scientists are promoting the use of joules rather than calories in the United States. However, kcal still predominates and is used throughout this text.]

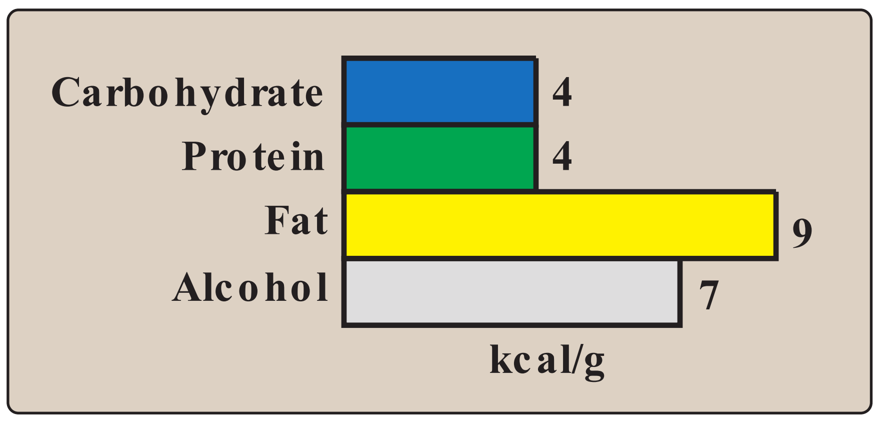


Figure 27.5

Average energy available from the major food components.

B. Use of food energy in the body

The energy generated by metabolism of the macronutrients is used for three energy-requiring processes that occur in the body: resting metabolic rate (RMR), physical activity, and thermic effect of food (formerly termed specific dynamic action). The number of calories expended by these processes in a 24-hour period is the total energy expenditure (TEE).

1. **Resting metabolic rate:** RMR is the energy expended by an individual in a resting, postabsorptive state. It represents the energy required to carry out the normal body functions, such as respiration, blood flow, and ion transport. [Note: A basal metabolic rate (BMR) is determined under more stringent environmental conditions. BMR can be determined by measuring O_2 consumed or CO_2 produced (indirect calorimetry). It also can be estimated using equations that include sex and age (BMR reflects lean muscle mass, which is highest in men and the young) as well as height and weight. A commonly used rough estimate is 1 kcal/kg/hour for men and 0.9 kcal/kg/hour for women. RMR is about 10% higher than the BMR.] In an adult, the 24-hour RMR, known as the resting energy expenditure (REE), is about 1,800 kcal for men (70 kg) and 1,300 kcal for women (50 kg). From 50%–70% of the TEE in sedentary individuals is attributable to the REE (Figure 27.6). [Note: Hospitalized individuals are commonly hypercatabolic, and an "injury factor" is included in calculating their TEE.]
2. **Physical activity:** Muscular activity provides the greatest variation in the TEE. The amount of energy consumed depends on the duration and intensity of the exercise. The daily expenditure of energy can be estimated by carefully recording the type and duration of all activities to determine a physical activity factor.

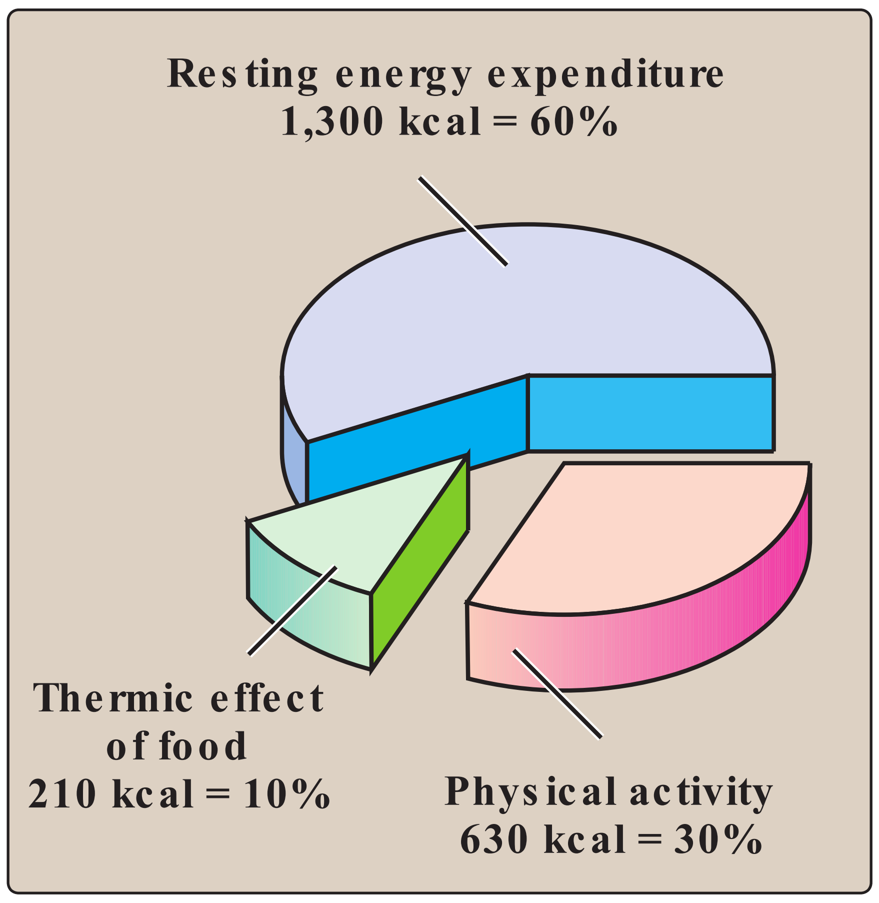


Figure 27.6

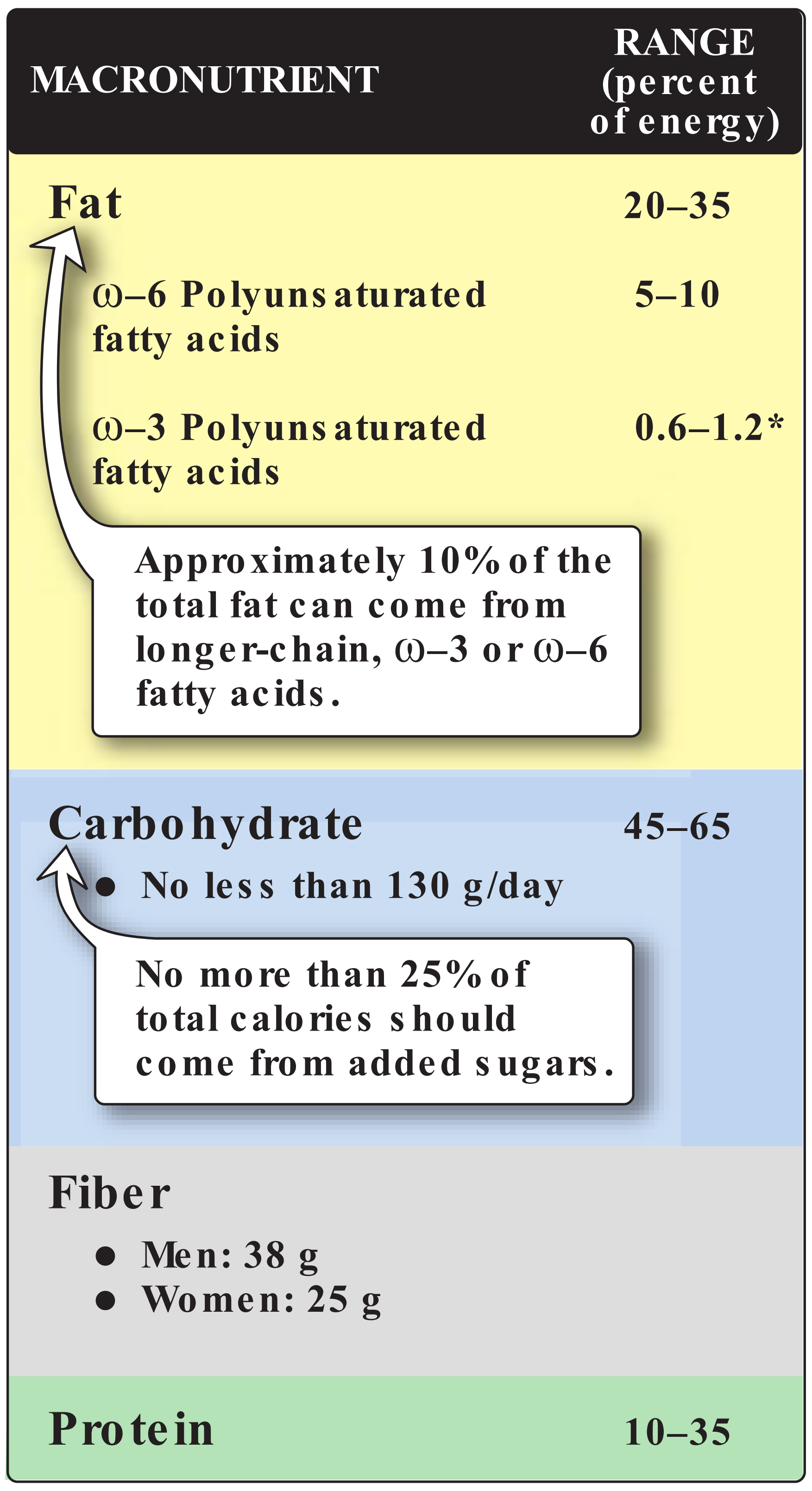
Estimated total energy expenditure in a healthy 20-year-old woman, 165 cm (5 feet, 4 inches) tall, weighing 50 kg (110 lb), and engaged in light activity.

In general, a sedentary person requires about 30%–50% more calories than the RMR (see Figure 27.6), whereas a highly active individual may require 100% or more calories above the RMR.

3. **Thermic effect of food:** The production of heat by the body increases as much as 30% above the resting level during the digestion and absorption of food. This is called the thermic effect of food, or diet-induced thermogenesis. The thermic response to food intake may amount to 5%–10% of the TEE.

IV. ACCEPTABLE MACRONUTRIENT DISTRIBUTION RANGES

Acceptable Macronutrient Distribution Ranges (AMDRs) are defined as a range of intakes for a particular macronutrient that is associated with reduced risk of chronic disease while providing adequate amounts of essential nutrients. The AMDR for adults is 45%–65% of their total calories from carbohydrates, 20%–35% from fat, and 10%–35% from protein (Figure 27.7). The biologic properties of dietary fat, carbohydrate, and protein are described below.



MACRONUTRIENT	RANGE (percent of energy)
Fat	20–35
ω–6 Polyunsaturated fatty acids	5–10
ω–3 Polyunsaturated fatty acids	0.6–1.2*
Approximately 10% of the total fat can come from longer-chain, ω–3 or ω–6 fatty acids.	
Carbohydrate	45–65
- No less than 130 g/day	
No more than 25% of total calories should come from added sugars.	
Fiber	
- Men: 38 g - Women: 25 g	
Protein	10–35

Figure 27.7
Acceptable macronutrient distribution ranges in adults. *A growing body of evidence suggests that higher levels of ω–3 polyunsaturated fatty acids provide protection against coronary heart disease.

V. DIETARY FATS

The incidence of a number of chronic diseases is significantly influenced by the kinds and amounts of nutrients consumed (Figure 27.8). Dietary fats most strongly influence the incidence of coronary heart disease (CHD), but evidence linking dietary fat and the risk for cancer or obesity is much weaker.

> Although earlier recommendations emphasized decreasing the total amount of fat and cholesterol in the diet, data now show that the type of fat is a more important risk factor than the total amount of fat consumed.

A. Plasma lipids and coronary heart disease

Plasma cholesterol may arise from the diet or from endogenous biosynthesis. In either case, cholesterol is transported between the tissues in combination with protein and phospholipids as lipoproteins.

1. **Low-density lipoprotein and high-density lipoprotein:** The level of plasma cholesterol is not precisely regulated but, rather, varies in response to the diet. Elevated levels of total cholesterol (hypercholesterolemia) result in an increased risk for CHD (Figure 27.9). A much stronger correlation exists between CHD and the level of cholesterol in low-density lipoproteins ([LDL-C] see p. 232). As LDL-C increases, CHD increases. In contrast, high levels of high-density lipoprotein cholesterol (HDL-C) have been associated with a decreased risk for heart disease (see p. 235). [Note: Elevated plasma triacylglycerol (TAG) is associated with CHD, but a causative relationship has yet to be demonstrated.] Abnormal levels of plasma lipids (dyslipidemias) act in combination with smoking, obesity, sedentary lifestyle, insulin resistance, and other risk factors to increase the risk of CHD.

2. **Benefits of lowering plasma cholesterol:** Dietary or drug treatment of hypercholesterolemia has been shown to be effective in decreasing LDL-C, increasing HDL-C, and reducing the risk for cardiovascular events. The diet-induced changes of plasma lipoprotein concentrations are modest, typically 10%–20%, whereas treatment with "statin" drugs decreases plasma cholesterol by 30%–60% (see p. 224). [Note: Dietary and drug treatment can lower TAGs.]

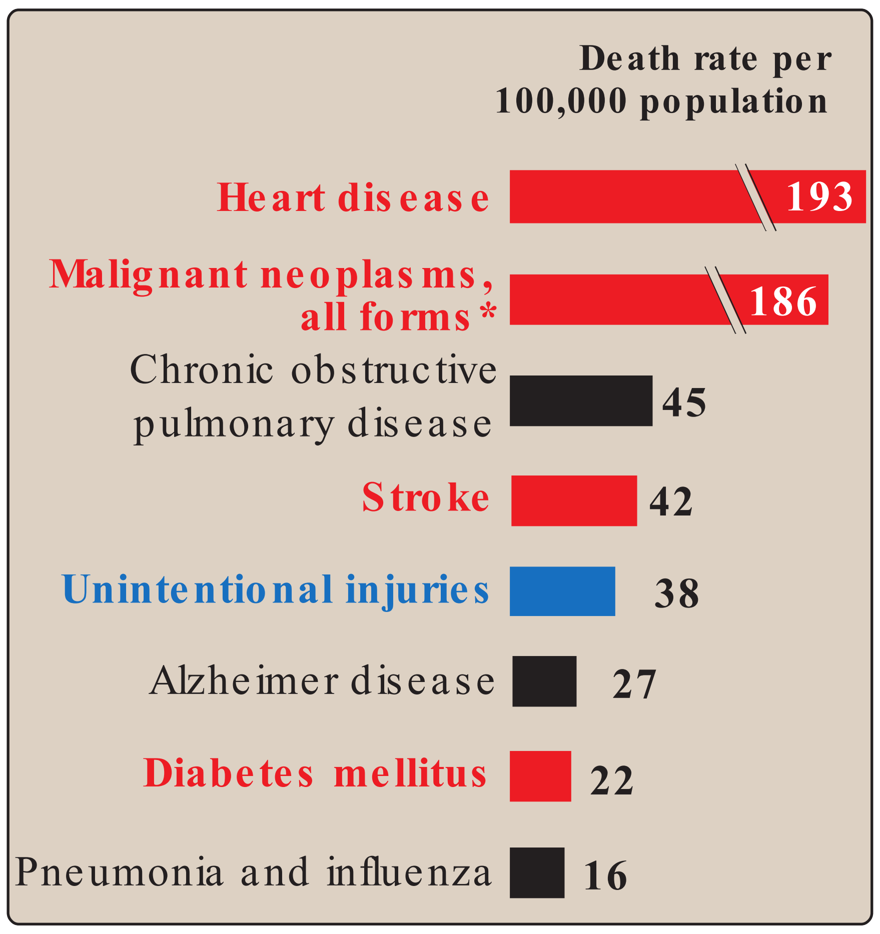


Figure 27.8
Influence of nutrition on some common causes of death in the United States in the year 2010. Red indicates causes of death in which the diet plays a significant role. Blue indicates causes of death in which excessive alcohol consumption plays a part. (*Diet plays a role in only some forms of cancer.)

B. Dietary fats and plasma lipids

TAGs are quantitatively the most important class of dietary fats. The influence of TAGs on blood lipids is determined by the chemical nature of their constituent fatty acids. The absence or presence and number of double bonds (saturated versus mono- and polyunsaturated), the location of the double bonds (w-6 versus w-3), and the cis versus trans configuration of the unsaturated fatty acids are the most important structural features that influence blood lipids.

1. **Saturated fat:** TAGs composed primarily of fatty acids whose hydrocarbon chains do not contain any double bonds are referred to as saturated fats. Consumption of saturated fats is positively associated with high levels of total plasma cholesterol and LDL-C and an increased risk of CHD. The main sources of saturated fatty acids are dairy and meat products and some vegetable oils, such as coconut and palm oils (a major source of fat in Latin America and Asia, although not in the United States; Figure 27.10). Most experts strongly advise limiting intake of saturated fats to less than 10% of total caloric intake.

Saturated fatty acids with carbon chain lengths of 14 (myristic) and 16 (palmitic) are most potent in increasing the serum cholesterol. Stearic acid (18 carbons, found in many foods including chocolate) has little effect on blood cholesterol.

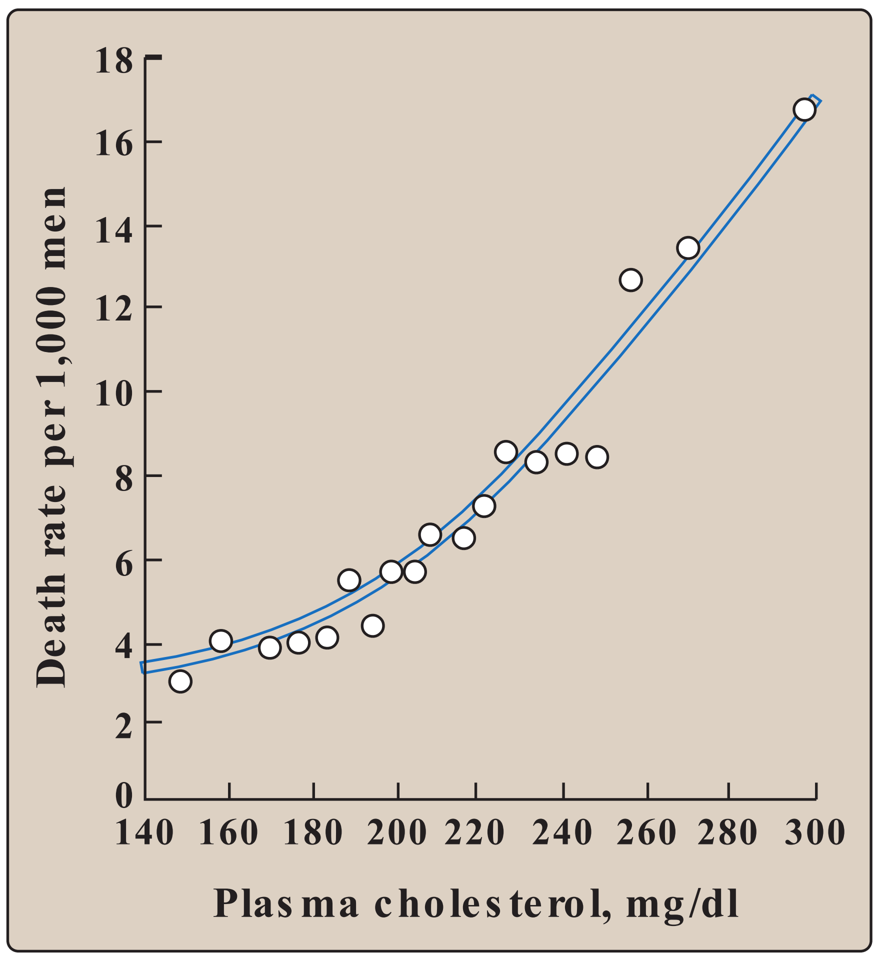


Figure 27.9
Correlation of the death rate from coronary heart disease with the concentration of plasma cholesterol. [Note: The data were obtained from a 6-year study of men with the death rate adjusted for age.]

2. **Monounsaturated fats:** TAGs containing primarily fatty acids with one double bond are referred to as monounsaturated fat. Unsaturated fatty acids are generally derived from vegetables and fish. When substituted for saturated fatty acids in the diet, monounsaturated fats lower both total plasma cholesterol and LDL-C but maintain or increase HDL-C. This ability of monounsaturated fats to favorably modify lipoprotein levels may explain, in part, the observation that Mediterranean cultures, with diets rich in olive oil (high in monounsaturated oleic acid), show a low incidence of CHD. It is recommended that monounsaturated fats account for about 50% of the total fat intake.

   a. **The Mediterranean diet:** The Mediterranean diet is an example of a diet rich in monounsaturated fatty acids, or MUFAs (from olive oil), and polyunsaturated fatty acids, or PUFAs (from fish oils and some nuts), but low in saturated fat. For example, Figure 27.11 shows the composition of the Mediterranean diet in comparison with both a Western diet similar to that consumed in the United States and a typical low-fat diet. The Mediterranean diet contains seasonally fresh food, with an abundance of plant

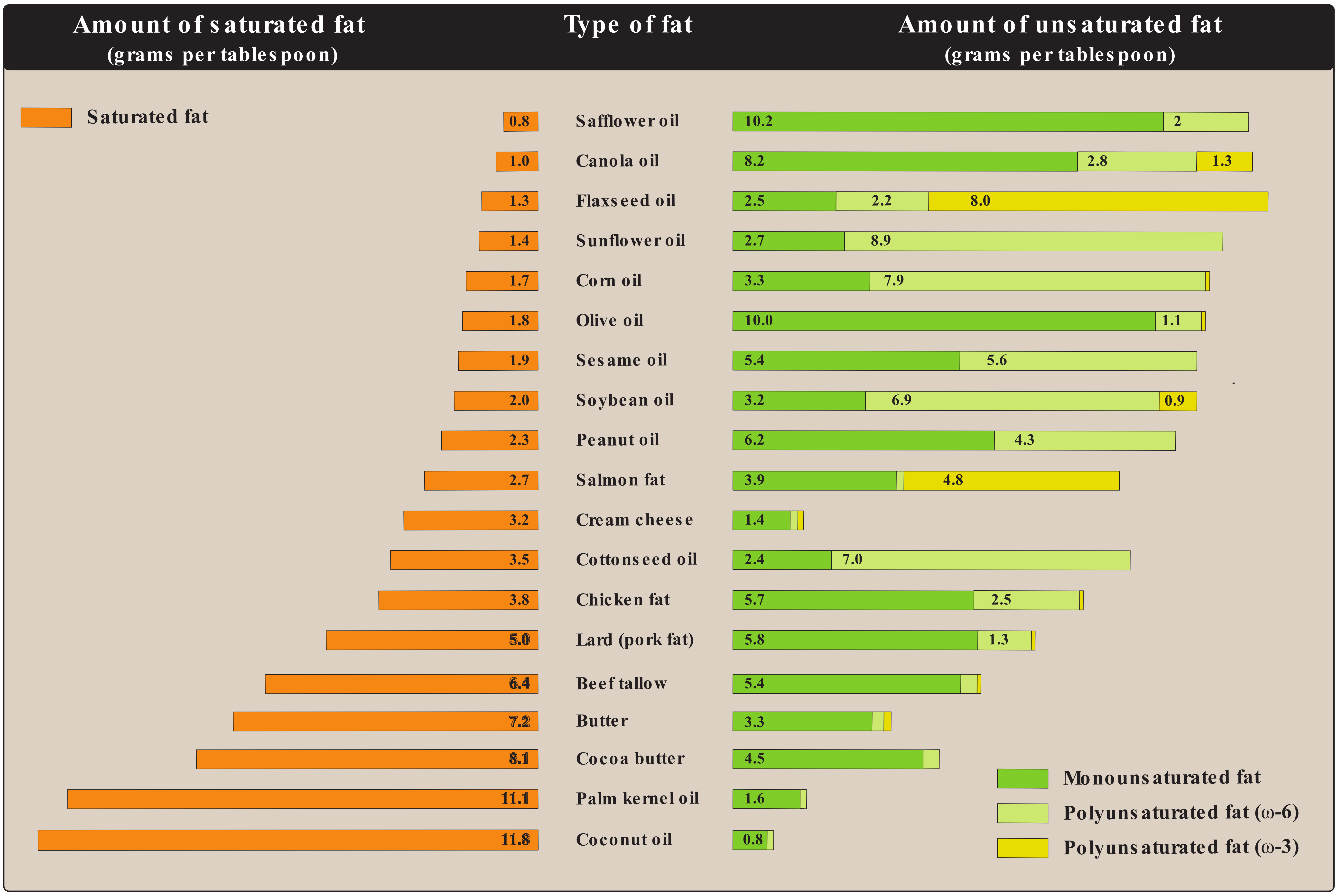


Figure 27.10
Compositions of commonly encountered dietary fats.

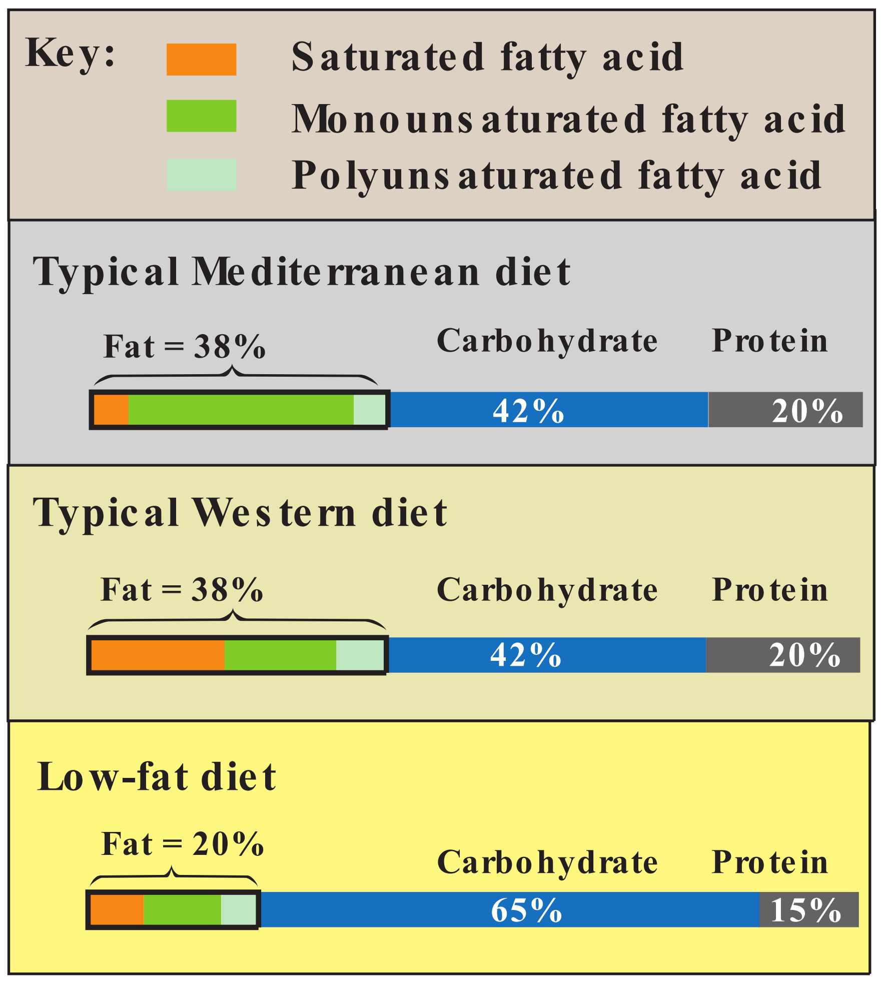
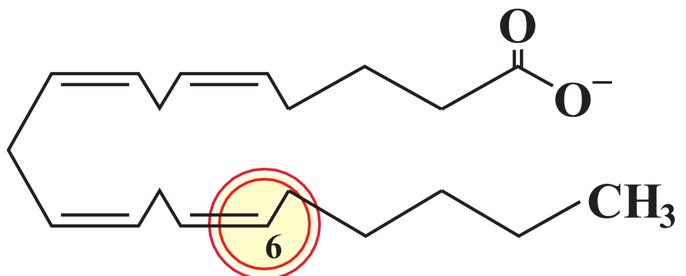


Figure 27.11
Composition of typical Mediterranean, Western, and low-fat diets.

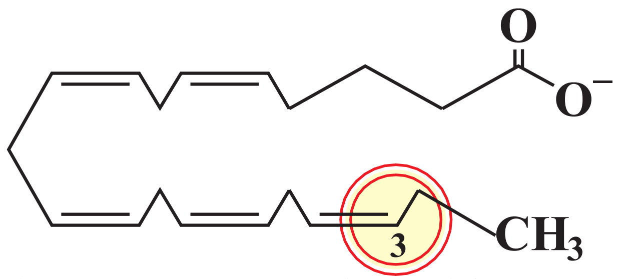
material, low amounts of red meat, and olive oil as the principal source of fat. The Mediterranean diet is associated with decreased serum total cholesterol and LDL-C, decreased TAGs, and increased HDL-C when compared with a typical Western diet higher in saturated fats.

3. **Polyunsaturated fats:** TAGs containing primarily fatty acids with more than one double bond are referred to as polyunsaturated fats. The effects of PUFAs on cardiovascular disease is influenced by the location of the double bonds within the molecule.

   a. **w-6 Fatty acids:** These are long-chain PUFAs, with the first double bond beginning at the sixth bond position when starting from the methyl (w) end of the fatty acid molecule. [Note: They are also called n-6 fatty acids (see p. 183).]



Arachidonic acid
(20:4, ω-6)
found in seed oils



Eicosapentaenoic acid (EPA)
(20:5, ω-3)
found in fish oils

Consumption of fats containing w-6 PUFAs, principally linoleic acid (18:2 [9,12]), obtained from vegetable oils, lowers plasma cholesterol when substituted for saturated fats. Plasma LDL-C is lowered, but HDL-C, which protects against CHD, is also lowered. The powerful benefits of lowering LDLs are only partially offset because of the decreased HDL. Nuts, avocados, olives, soybeans, and various oils, including sunflower, cottonseed, and corn oil, are common sources of these fatty acids (see Figure 27.10). The AMDR for linoleic acid is 5%–10%. [Note: The lower recommendation for PUFAs relative to MUFAs is because of concern that free radical–mediated oxidation (peroxidation) of PUFAs may lead to deleterious products.]

b. w**-3 Fatty acids:** These are long-chain PUFAs, with the first double bond beginning at the third bond position from the methyl (w) end. Dietary w-3 PUFAs suppress cardiac arrhythmias, reduce serum TAGs, decrease the tendency for thrombosis, lower blood pressure, and substantially reduce risk of cardiovascular mortality (Figure 27.12), but they have little effect on LDL-C or HDL-C levels. Evidence suggests that they have anti-inflammatory effects. The w-3 PUFAs, principally α-linolenic acid, 18:3(9,12,15), are found in plant oils, such as flaxseed and canola, and some nuts. The AMDR for α-linolenic acid is 0.6%–1.2%. Fish oil contains the long-chain w-3 docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Two fatty fish (for example, salmon) meals per week are recommended. For patients with documented CHD, 1 g/day of fish oils is recommended, while 2–4 g/day is prescribed to lower TAGs. [Note: The ω-3 long-chain PUFAs are included in infant formulas to promote brain development.] Linoleic and α-linolenic acids are essential fatty acids (EFAs) required for membrane fluidity and synthesis of eicosanoids (see p. 213). EFA deficiency is characterized by scaly dermatitis as a result of the depletion of skin ceramides with long-chain fatty acids (see p. 206).]

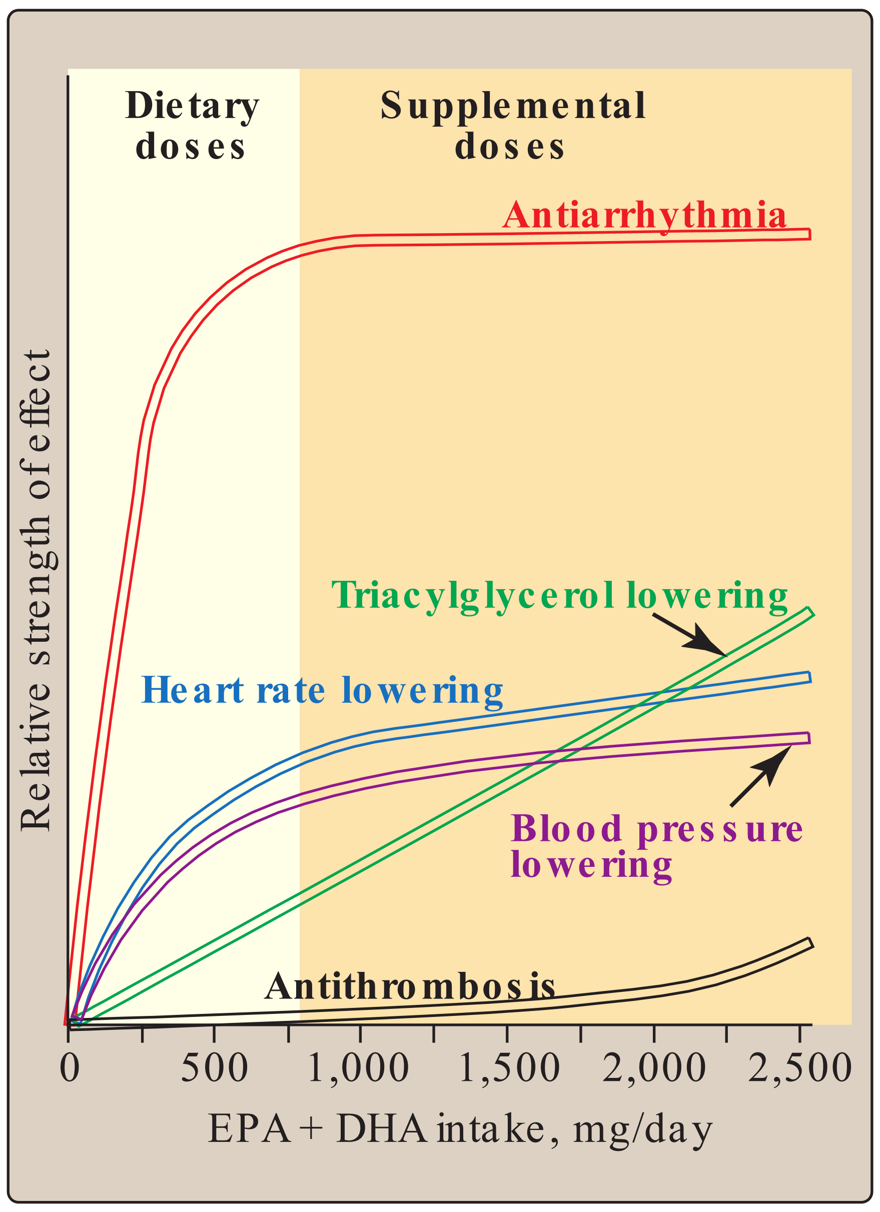


Figure 27.12
Dose responses of physiologic effects of fish oil intake. EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.

4. **Trans fatty acids:** Trans fatty acids (Figure 27.13) are chemically classified as unsaturated fatty acids but behave more like saturated fatty acids in the body because they elevate serum LDL-C (but not HDL-C), and they increase the risk of CHD. Trans fatty acids do not occur naturally in plants but occur in small amounts in animals. However, trans fatty acids are formed during the hydrogenation of liquid vegetable oils (for example, in the manufacture of margarine and partially hydrogenated vegetable oil). Trans fatty acids are a major component of many commercial baked goods, such as cookies and cakes, and most deep-fried foods. Many manufacturers have reformulated their products to be free of trans fats. Starting in 2006, the U.S. Food and Drug Administration requires that Nutrition Facts labels portray the trans fat content of packaged food. Some municipalities, such as New York City, have banned the use of trans fats in restaurants.

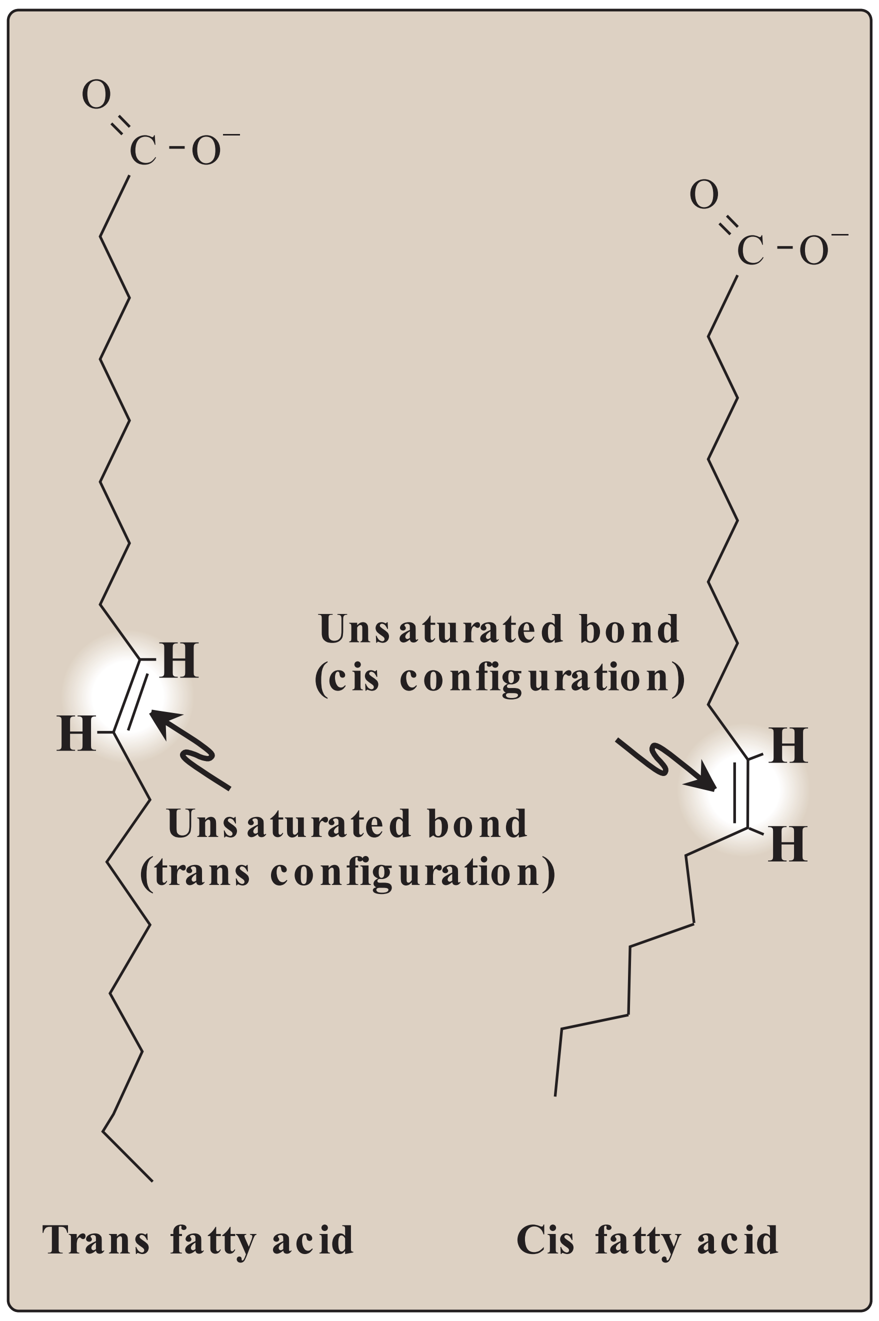

O
C-O⁻
H
H
Unsaturated bond (trans configuration)
O
C-O⁻
Unsaturated bond (cis configuration)
H
H
Trans fatty acid
Cis fatty acid

Figure 27.13
Structure of cis and trans fatty acids.

5. **Dietary cholesterol:** Cholesterol is found only in animal products. The effect of dietary cholesterol on plasma cholesterol (Figure 27.14) is less important than the amount and types of fatty acids consumed. Cholesterol consumption should be no more than 300 mg/day.

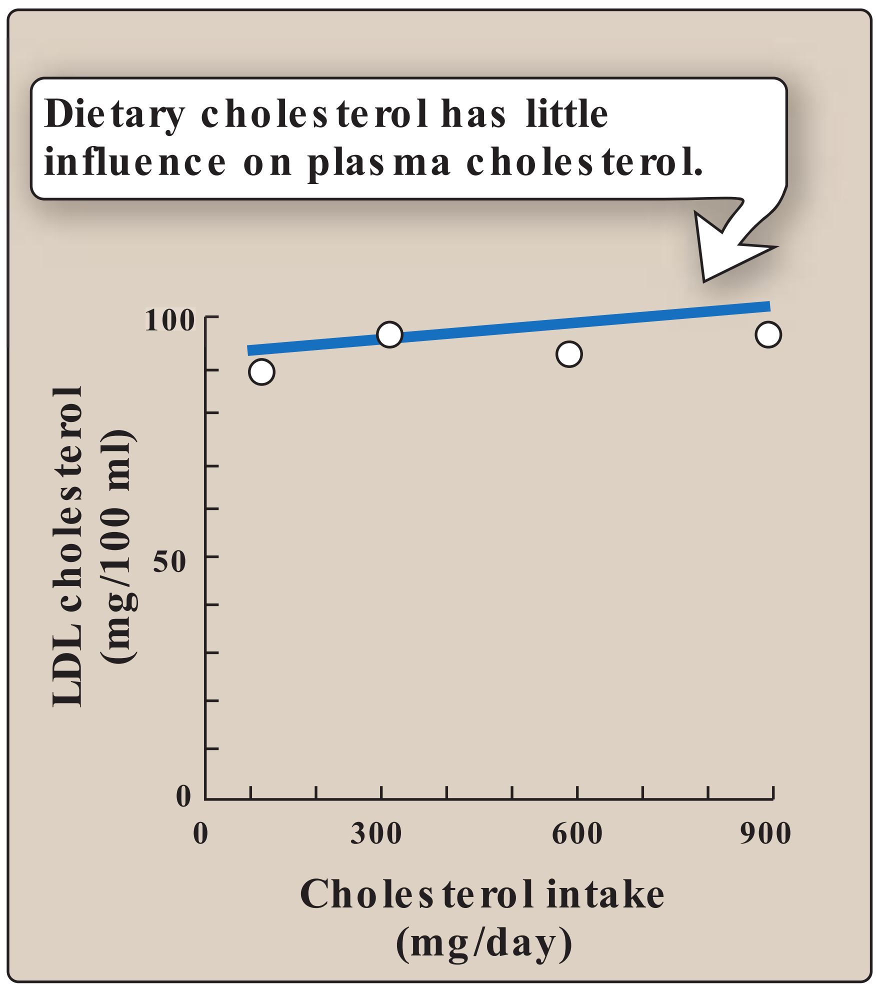


Figure 27.14
Response of plasma low-density lipoprotein (LDL) concentrations to an increase in dietary cholesterol intake.

C. Other dietary factors affecting coronary heart disease

Moderate consumption of alcohol (up to 1 drink a day for women and up to 2 drinks a day for men) decreases the risk of CHD, because there is a positive correlation between moderate alcohol consumption and the plasma concentration of HDL-C. However, because of the potential dangers of alcohol abuse, health professionals are reluctant to recommend increased alcohol consumption to their patients. Red wine may provide cardioprotective benefits in addition to those resulting from its alcohol content (for example, red wine contains phenolic compounds that inhibit lipoprotein oxidation; see p. 235). [Note: These antioxidants are also present in raisins and grape juice.] Figure 27.15 summarizes the effects of dietary fats.

VI. DIETARY CARBOHYDRATES

The primary role of dietary carbohydrate is to provide energy. Although caloric intake in the United States has shown a modest increase since 1971, the incidence of obesity has dramatically increased (see p. 349). During this same period, carbohydrate consumption has significantly increased (as fat consumption decreased), leading some observers to link obesity with carbohydrate consumption. However, obesity has also been related to increasingly inactive lifestyles and to calorie-dense foods served in expanded portion size. Carbohydrates are not inherently fattening.

A. Classification of carbohydrates

Dietary carbohydrates are classified as simple sugars (monosaccharides and disaccharides), complex sugars (polysaccharides), and fiber.

1. **Monosaccharides:** Glucose and fructose are the principal monosaccharides found in food. Glucose is abundant in fruits, sweet corn, corn syrup, and honey. Free fructose is found together with free glucose and sucrose in honey and fruits (for example, apples).

TYPE OF FAT	METABOLIC EFFECTS	EFFECTS ON DISEASE PREVENTION
Trans fatty acid	↑ LDL ↓ HDL	↑ Incidence of coronary heart disease
Saturated fatty acid	↑ LDL Little effect on HDL	↑ Incidence of coronary heart disease; may increase risk of prostate, colon cancer
Monounsaturated fatty acid	↓ LDL Maintain or increase HDL	↓ Incidence of coronary heart disease
Polyunsaturated fatty acids ω–6	↓ LDL ↓ HDL Provide arachidonic acid, which is an important precursor of prostaglandins and leukotrienes	↓ Incidence of coronary heart disease
Polyunsaturated fatty acids ω–3	Little effect on LDL Little effect on HDL Suppress cardiac arrhythmias, reduce serum triacylglycerols, decrease the tendency for thrombosis, lower blood pressure	↓ Incidence of coronary heart disease ↓ Risk of sudden cardiac death

Figure 27.15
Effects of dietary fats. LDL = low-density lipoprotein; HDL = high-density lipoprotein.

a. **High-fructose corn syrup:** High-fructose corn syrups (HFCSs) are corn syrups that have undergone enzymatic processing to convert their glucose into fructose and have then been mixed with pure corn syrup (100% glucose) to produce a desired sweetness. In the United States, HFCS 55 (containing 55% fructose and 42% glucose) is commonly used as a substitute for sucrose in beverages, including soft drinks, with HFCS 42 used in processed foods. The composition and metabolism of HFCS and sucrose are similar, the major difference being that HFCS is ingested as a mixture of monosaccharides (Figure 27.16). Most studies have shown no significant difference between sucrose and HFCS syrup meals in either postprandial glucose or insulin responses. [Note: The rise in the use of HFCS parallels the rise in obesity, but a causal relationship has not been demonstrated.]

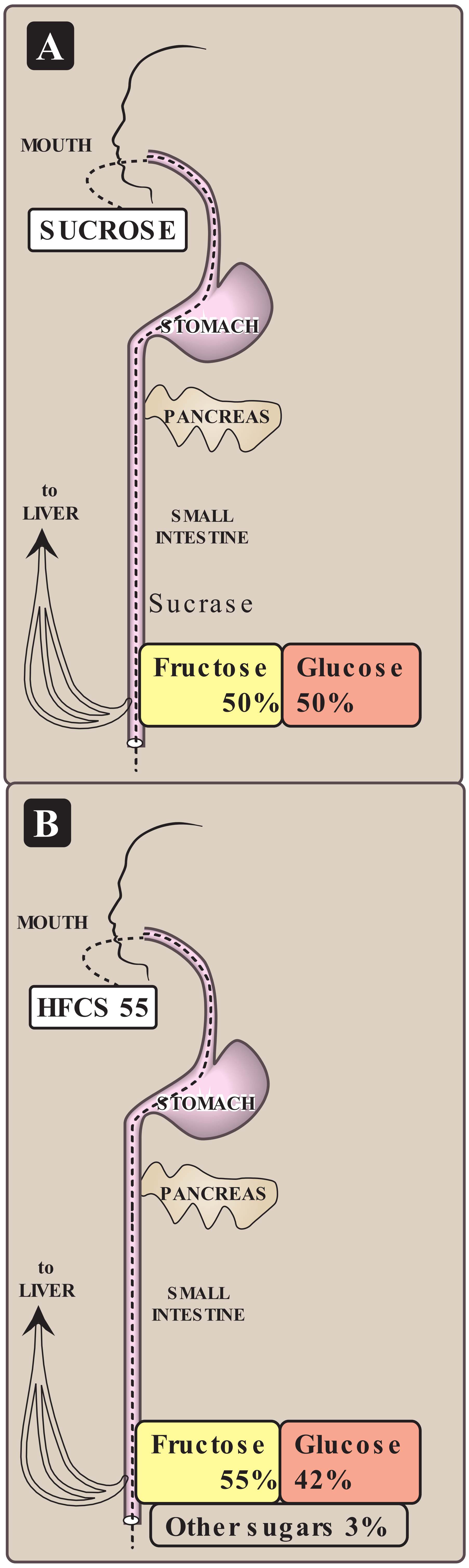


Figure 27.16
Digestion of high-fructose corn syrup (HFCS) 55 or sucrose leads to absorption of glucose plus fructose.

2. **Disaccharides:** The most abundant disaccharides are sucrose (glucose + fructose), lactose (glucose + galactose), and maltose (glucose + glucose). Sucrose is ordinary "table sugar" and is abundant in molasses and maple syrup. Lactose is the principal sugar found in milk. Maltose is a product of enzymic digestion of polysaccharides. It is also found in significant quantities in beer and malt liquors. The term "sugar" refers to monosaccharides and disaccharides. "Added sugars" are those sugars and syrups (such as HFCSs) added to foods during processing or preparation. [Note: Fructose is 1.7 times sweeter than sucrose.]

3. **Polysaccharides:** Complex carbohydrates are polysaccharides (most often polymers of glucose) that do not have a sweet taste. Starch is an example of a complex carbohydrate that is found in abundance in plants. Common sources include wheat and other grains, potatoes, dried peas and beans, and vegetables.

4. **Fiber:** Dietary fiber is defined as the nondigestible, nonstarch carbohydrates and lignin (a noncarbohydrate polymer of aromatic alcohols) present intact in plants. Soluble fiber is the edible parts of plants that is resistant to digestion and absorption in the human small intestine but is completely or partially fermented by bacteria to short-chain fatty acids in the large intestine. Insoluble fiber passes through the digestive track largely intact. Dietary fiber provides little energy but has several beneficial effects. First, it adds bulk to the diet (Figure 27.17). Fiber can absorb 10–15 times its own weight in water, drawing fluid into the lumen of the intestine and increasing bowel motility and promoting normal laxation. Soluble fiber delays gastric emptying and can result in a sensation of fullness. This delayed emptying also results in reduced peaks of blood glucose following a meal. Second, consumption of soluble fiber has now been shown to lower LDL-C levels by increasing fecal bile acid excretion and interfering with bile acid absorption (see p. 225). For example, diets rich (25–50 g/day) in the soluble fiber oat bran are associated with a modest, but significant, reduction in risk for CHD by lowering total cholesterol and LDL-C levels. Also, fiber-rich diets decrease the risk for constipation, hemorrhoids, and diverticulosis. The AI for dietary fiber is 25 g/day for women and 38 g/day for men. However, most American diets are far lower in fiber at approximately 15 g/day. [Note: "Functional fiber" is the term used for isolated fiber that has proven health benefits

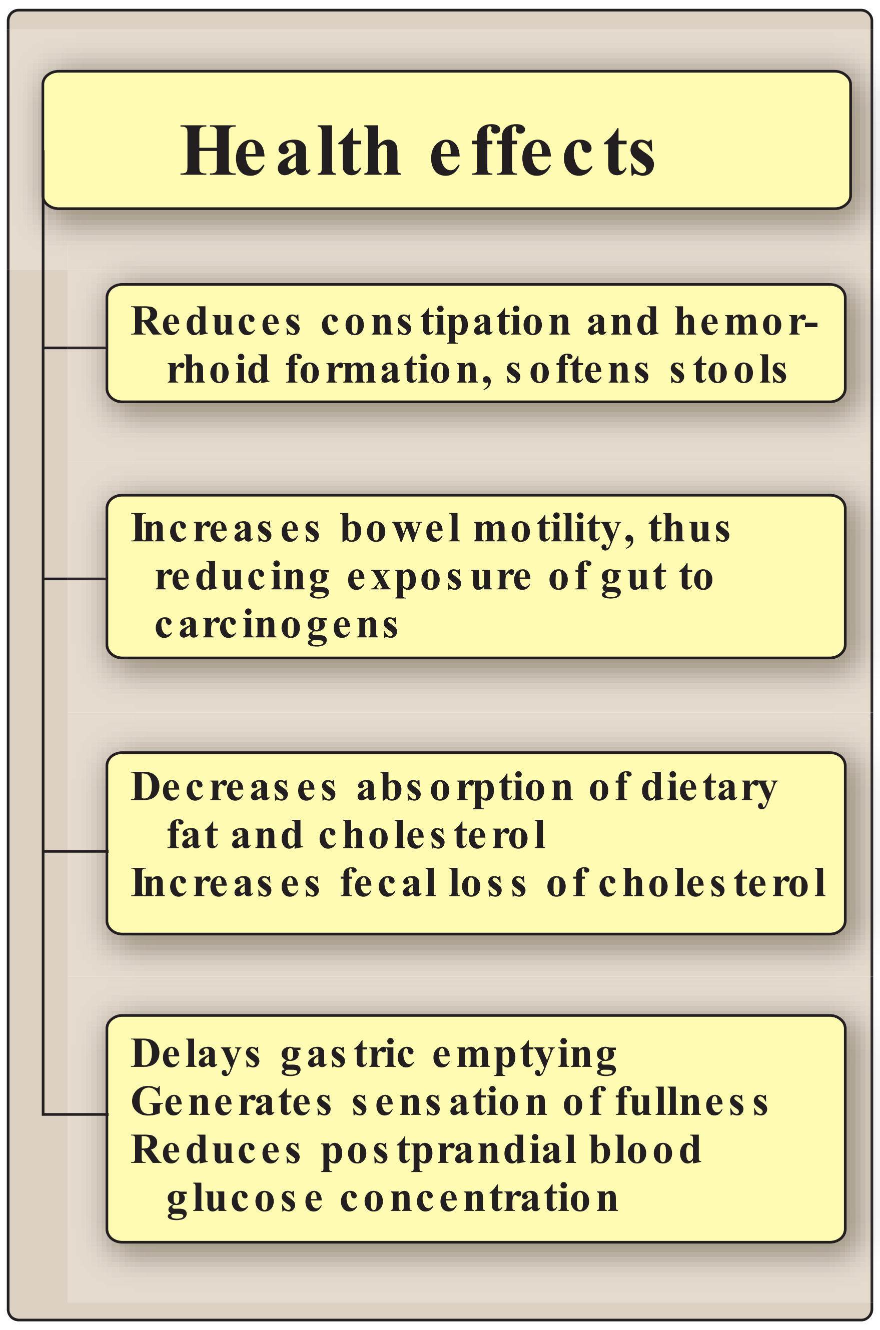

Health effects
Reduces constipation and hemorrhoid formation, softens stools
Increases bowel motility, thus reducing exposure of gut to carcinogens
Decreases absorption of dietary fat and cholesterol
Increases fecal loss of cholesterol
Delays gastric emptying
Generates sensation of fullness
Reduces postprandial blood glucose concentration

Figure 27.17
Actions of dietary fiber.

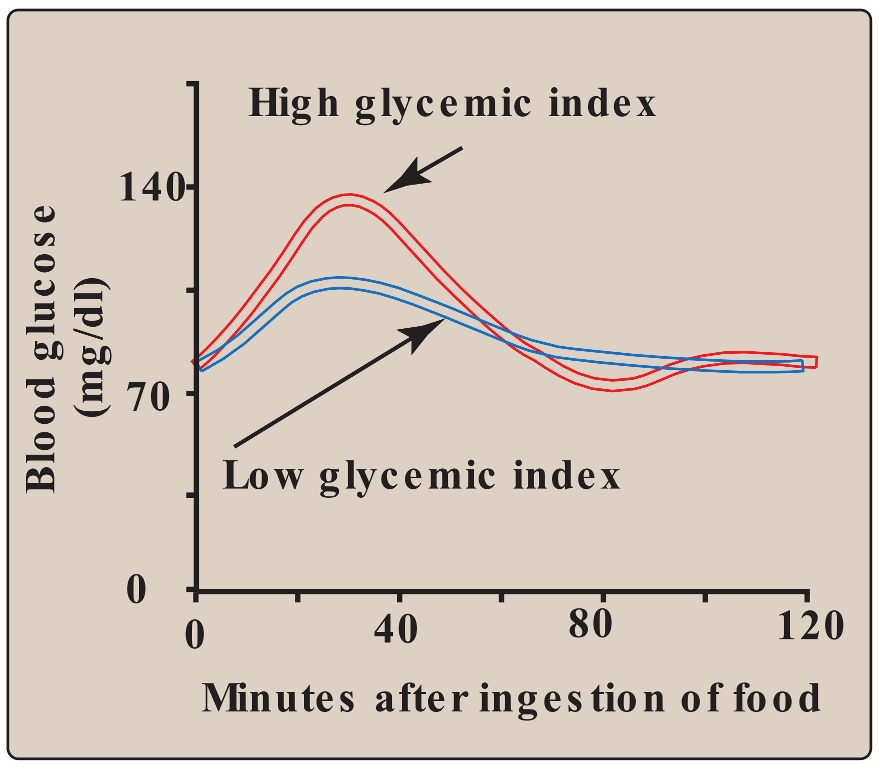


Figure 27.18
Blood glucose concentrations following ingestion of food with low or high glycemic index.

such as commercially available fiber supplements. Total fiber is the sum of dietary fiber and functional fiber.]

B. Dietary carbohydrate and blood glucose

Some carbohydrate-containing foods produce a rapid rise followed by a steep fall in blood glucose concentration, whereas others result in a gradual rise followed by a slow decline. Thus, they differ in their glycemic response (GR). [Note: Fiber blunts the GR.] The glycemic index (GI) quantitates these differences in the time course of postprandial glucose concentrations (Figure 27.18). GI is defined as the area under the blood glucose curves seen after ingestion of a meal with carbohydrate-rich food, compared with the area under the blood glucose curve observed after a meal consisting of the same amount (50 g) of carbohydrate either as glucose or white bread. The clinical importance of the GI is unresolved, but evidence suggests that a low-GI diet improves glycemic control in diabetic individuals. Food with a low GI tends to create a sense of satiety over a longer period of time and may be helpful in limiting caloric intake. [Note: How much a typical serving size of a food raises blood glucose is referred to as the glycemic load (GL). A food (for example, carrots) can have a high GI and a low GL.]

C. Requirements for carbohydrate

Carbohydrates are not essential nutrients, because the carbon skeletons of most amino acids can be converted into glucose (see p. 261). However, the absence of dietary carbohydrate leads to ketone body production (see p. 262) and degradation of body protein whose constituent amino acids provide carbon skeletons for gluconeogenesis (see p. 118). The RDA for carbohydrate is set at 130 g/day for adults and children, based on the amount of glucose used by carbohydrate-dependent tissues, such as the brain and erythrocytes. However, this level of intake is usually exceeded to meet energy needs. Adults should consume 45%–65% of their total calories from carbohydrates. It is recommended that added sugar represent no more than 25% of total energy because of concerns that sugar may displace nutrient-rich foods from the diet, potentially leading to deficiencies of certain micronutrients.

D. Simple sugars and disease

There is no direct evidence that the consumption of simple sugars is harmful. Contrary to folklore, diets high in sucrose do not lead to diabetes or hypoglycemia. Also contrary to popular belief, carbohydrates are not inherently fattening. They yield 4 kcal/g (the same as protein and less than one half that of fat; see Figure 27.5) and result in fat synthesis only when consumed in excess of the body's energy needs. However, there is an association between sucrose consumption and dental caries, particularly in the absence of fluoride treatment.

VII. DIETARY PROTEIN

Humans have no dietary requirement for protein per se, but the protein in food does provide essential amino acids (see Figure 20.2, p. 262). Nine of the 20 amino acids needed for the synthesis of body proteins are essential (that is, they cannot be synthesized in humans).

A. Quality of proteins

The quality of a dietary protein is a measure of its ability to provide the essential amino acids required for tissue maintenance. Most government agencies have adopted the Protein Digestibility–Corrected Amino Acid Score (PDCAAS) as the standard by which to evaluate protein quality. PDCAAS is based on the profile of essential amino acids after correcting for the digestibility of the protein. The highest possible score under these guidelines is 1.00. This amino acid score provides a method to balance intakes of poorer-quality proteins with high-quality dietary proteins.

Source	PDCAAS value
Animal proteins	
Egg	1.00
Milk protein	1.00
Beef/poultry/fish	0.82–0.92
Gelatin	0.08
Plant proteins	
Soybean protein	1.00
Kidney beans	0.68
Whole wheat bread	0.40

Figure 27.19
Relative quality of some common dietary proteins. PDCAAS = protein digestibility–corrected amino acid score.

1. **Proteins from animal sources:** Proteins from animal sources (meat, poultry, milk, and fish) have a high quality because they contain all the essential amino acids in proportions similar to those required for synthesis of human tissue proteins (Figure 27.19), and they are more readily digested. [Note: Gelatin prepared from animal collagen is an exception. It has a low biologic value as a result of deficiencies in several essential amino acids.]

2. **Proteins from plant sources:** Proteins from plant sources have a lower quality than do animal proteins. However, proteins from different plant sources may be combined in such a way that the result is equivalent in nutritional value to animal protein. For example, wheat (lysine deficient but methionine rich) may be combined with kidney beans (methionine poor but lysine rich) to produce an improved biologic value. Therefore, eating foods with different amino acids during the day can result in a dietary combination with a higher biologic value than either of the component proteins (Figure 27.20). [Note: Animal proteins can also complement the biologic value of plant proteins.]

B. Nitrogen balance

Nitrogen balance occurs when the amount of nitrogen consumed equals that of the nitrogen excreted in the urine (primarily as urinary urea nitrogen, or UUN), sweat, and feces. Most healthy adults are normally in nitrogen balance.

1. **Positive nitrogen balance:** This occurs when nitrogen intake exceeds nitrogen excretion. It is observed during situations in which tissue growth occurs, for example, in childhood, pregnancy, or during recovery from an emaciating illness.

2. **Negative nitrogen balance:** This occurs when nitrogen loss is greater than nitrogen intake. It is associated with inadequate dietary protein; lack of an essential amino acid; or during physiologic stresses, such as trauma, burns, illness, or surgery.

Nitrogen (N) balance can be determined by the formula, N balance = protein N intake (g/24 hrs) – (UUN + 4 g), where 4 g accounts for urinary loss in forms other than UUN plus loss in skin and feces. Alternatively, protein balance = protein intake (g/24 hrs) – [(UUN + 4 g) x 6.25] can be used because there are, on average, 6.25 g N in 1 g protein.

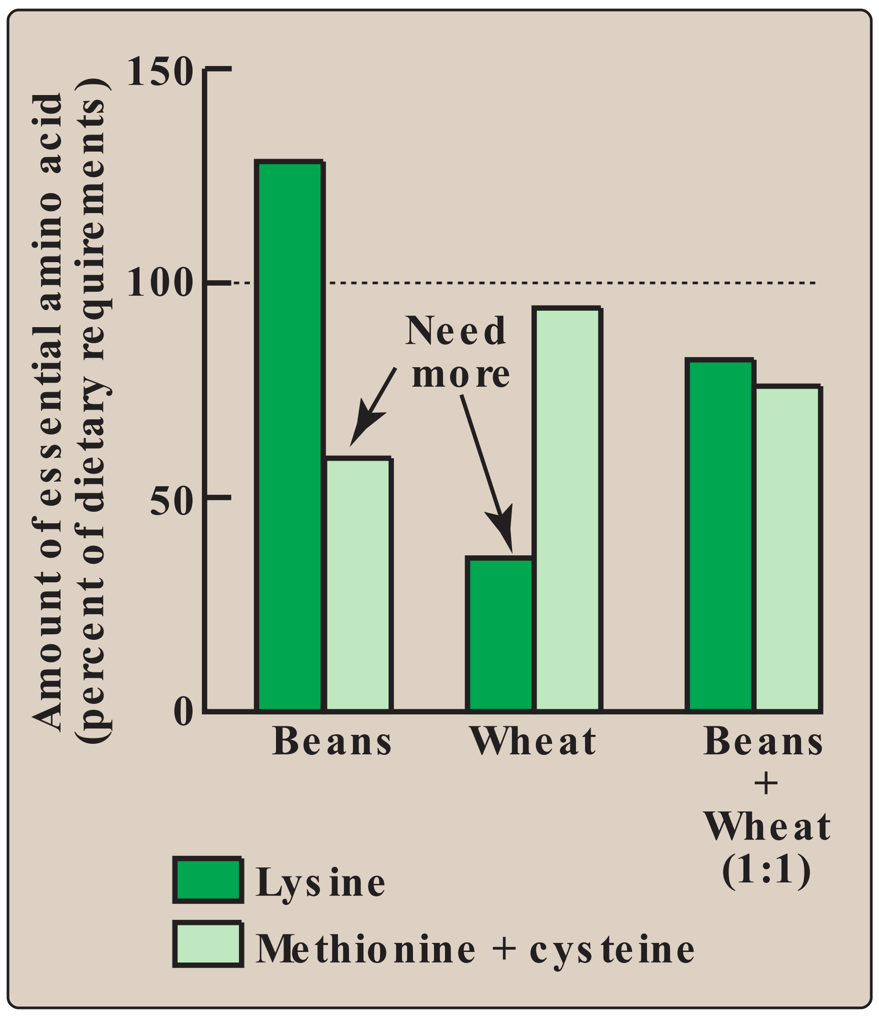


Figure 27.20
Combining two incomplete proteins that have complementary amino acid deficiencies results in a mixture with a higher biologic value.

C. Requirement for protein in humans

The amount of dietary protein required in the diet varies with its biologic value. The greater the proportion of animal protein included in the diet, the less protein is required. The RDA for protein is computed for proteins of mixed biologic value at 0.8 g/kg of body weight for adults, or about 56 g of protein for a 70-kg individual. People who exercise strenuously on a regular basis may benefit from extra protein to maintain muscle mass, and a daily intake of about 1 g/kg has been recommended for athletes. Women who are pregnant or lactating require up to 30 g/day in addition to their basal requirements. To support growth, infants should consume 2 g/kg/day.

1. **Consumption of excess protein:** There is no physiologic advantage to the consumption of more protein than the RDA. Protein consumed in excess of the body's needs is deaminated, and the resulting carbon skeletons are metabolized to provide energy or acetyl coenzyme A for fatty acid synthesis. When excess protein is eliminated from the body as urinary nitrogen, it is often accompanied by increased urinary calcium, thereby increasing the risk of nephrolithiasis and osteoporosis.

2. **The protein-sparing effect of carbohydrate:** The dietary protein requirement is influenced by the carbohydrate content of the diet. When the intake of carbohydrates is low, amino acids are deaminated to provide carbon skeletons for the synthesis of glucose that is needed as a fuel by the central nervous system. If carbohydrate intake is less than 130 g/day, substantial amounts of protein are metabolized to provide precursors for gluconeogenesis. Therefore, carbohydrate is considered to be "protein-sparing," because it allows amino acids to be used for repair and maintenance of tissue protein rather than for gluconeogenesis.

D. Protein-energy (calorie) malnutrition

In developed countries, protein-energy malnutrition (PEM) is most commonly seen in patients with medical conditions that decrease appetite or alter how nutrients are digested or absorbed or in hospitalized patients with major trauma or infections. [Note: Such highly catabolic patients frequently require intravenous (parenteral) or

CHARACTERISTIC	KWASHIORKOR	MARASMUS
Weight for age (% expected)	60–80	<60
Weight for height	Normal or decreased	Markedly decreased
Edema	Present	Absent
Mood	Irritable when picked up; apathetic when left alone	Alert, irritable
Appetite	Poor	Good

Figure 27.21
Features of protein-energy malnutrition in children.

tube-based (enteral) administration of nutrients.] PEM may also be seen in children or the elderly who are malnourished. In developing countries, an inadequate intake of protein and/or energy is the primary cause of PEM. Affected individuals show a variety of symptoms, including a depressed immune system with a reduced ability to resist infection. Death from secondary infection is common. PEM is a spectrum of degrees of malnutrition, and two extreme forms are kwashiorkor and marasmus (Figure 27.21). [Note: PEM is also referred to as protein-energy undernutrition (PEU).]

1. **Kwashiorkor:** Kwashiorkor occurs when protein deprivation is relatively greater than the reduction in total calories. Protein deprivation is associated with severely decreased synthesis of visceral protein. Kwashiorkor is commonly seen in developing countries in children after weaning at about age 1 year, when their diet consists predominantly of carbohydrates. Typical symptoms include stunted growth, skin lesions, depigmented hair, anorexia, enlarged fatty liver, edema, and decreased serum albumin concentration. Edema results from the lack of adequate blood proteins, primarily albumin, to maintain the distribution of water between blood and tissues. It may mask muscle loss. Therefore, chronic malnutrition is reflected in the level of serum albumin. [Note: Because caloric intake from carbohydrates may be adequate, insulin levels suppress lipolysis and proteolysis. Kwashiorkor is nonadapted malnutrition.]

Cachexia, a wasting disorder characterized by loss of appetite and muscle atrophy (with or without increased lipolysis) that cannot be reversed by conventional nutritional support, is seen with a number of chronic diseases, such as cancer and chronic pulmonary and renal disease. It is associated with decreased treatment tolerance and response and decreased survival time.

2. **Marasmus:** Marasmus occurs when calorie deprivation is relatively greater than the reduction in protein. It usually occurs in developing countries in children younger than age 1 year when breast milk is supplemented with watery gruels of native cereals that are usually deficient in both protein and calories. Typical symptoms include arrested growth, extreme muscle wasting and loss of subcutaneous fat (emaciation), weakness, and anemia (Figure 27.22). Individuals with marasmus do not show the edema observed in kwashiorkor.

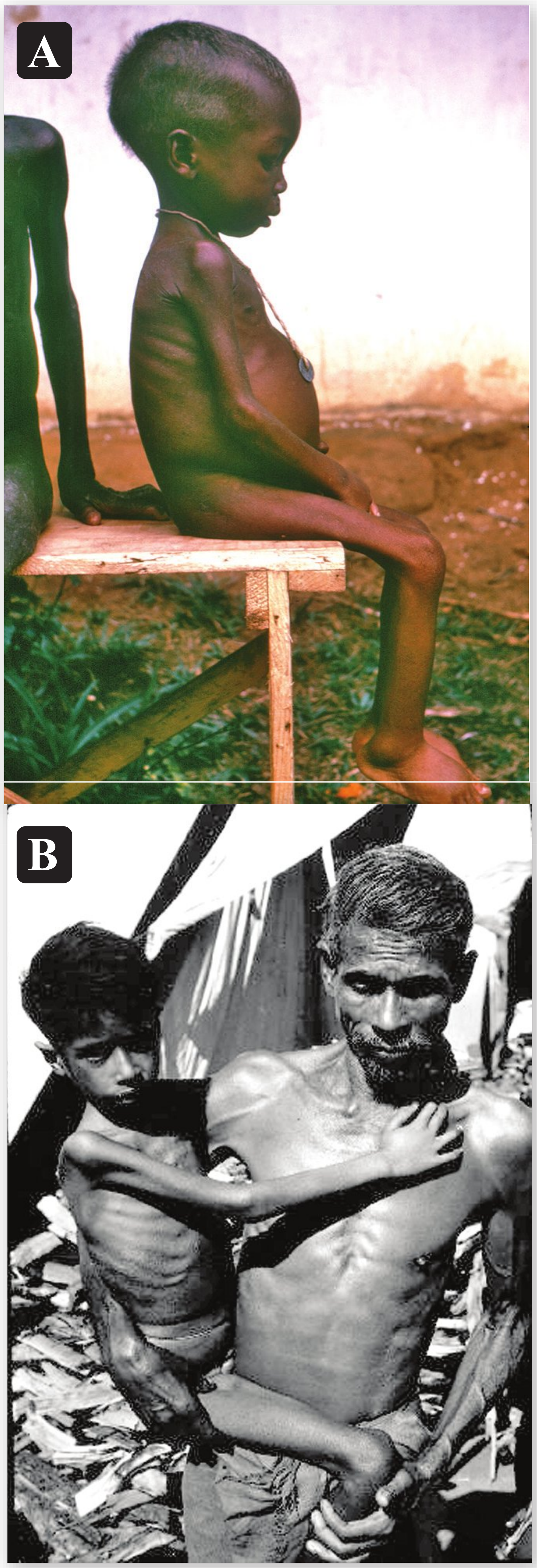


Figure 27.22
A.Listless child with kwashiorkor. Note the swollen belly.
B.Child suffering with marasmus.

VIII. CHAPTER SUMMARY

The **Dietary Reference Intakes** (**DRIs**) provide estimates of the amounts of nutrients required to prevent deficiencies and maintain optimal health and growth. It consists of the **Estimated Average Requirement** (**EAR)**, the average daily nutrient intake level estimated to meet the requirement of 50% of the healthy individuals in a particular life stage (age) and gender group; the **Recommended Dietary Allowance** (**RDA**), the average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97%–98%) individuals in a life stage and gender group; the **Adequate Intake** (**AI**), which is set instead of an RDA if sufficient scientific evidence is not available to calculate the RDA; and the **Tolerable Upper Intake Level** (**UL**), the highest average daily nutrient intake level that is likely to pose no risk of adverse health effects to almost all individuals in the general population. The energy generated by the metabolism of the **macronutrients** is used for three energy-requiring processes that occur in the body: **resting metabolic rate**, **physical activity, and thermic effect of food. Acceptable Macronutrient Distribution Ranges** (**AMDR**) are defined as the ranges of intake for a particular macronutrient that are associated with reduced risk of chronic disease while providing adequate amounts of essential nutrients. Adults should consume **45%–65%** of their **total calories** from **carbohydrates**, **20%–35%** from **fat**, and **10%–35%** from **protein** (Figure 27.23). Elevated levels of cholesterol in low-density lipoproteins (LDL-C) result in increased risk for **cardiovascular disease**. In contrast, high levels of cholesterol in high-density lipoproteins (HDL-C) have been associated with a decreased risk for heart disease. Dietary or drug treatment of **hypercholesterolemia** is effective in decreasing LDL-C, increasing HDL-C, and reducing the risk for cardiovascular events. Consumption of **saturated fats** is strongly associated with high levels of total plasma and LDL-C. When substituted for saturated fatty acids in the diet, **monounsaturated fats** lower both total plasma cholesterol and LDL-C but maintain or increase HDL-C. Consumption of fats containing **w-6 polyunsaturated fatty acids** lowers plasma LDL-C, but HDL-C, which protects against coronary heart disease, is also lowered. Dietary **w-3 polyunsaturated fats** suppress cardiac arrhythmias and reduce serum triacylglycerols, decrease the tendency for thrombosis, and substantially reduce the risk of cardiovascular mortality. **Carbohydrates** provide **energy** and **fiber** to the diet. When they are consumed as part of a diet in which caloric intake is equal to energy expenditure, they do not promote obesity. Dietary **protein** provides **essential amino acids**. The **quality of a protein** is a measure of its ability to provide the essential amino acids required for tissue maintenance. Proteins from animal sources, in general, have a higher-quality protein than that derived from plants. However, proteins from different plant sources may be combined in such a way that the result is equivalent in nutritional value to animal protein. **Positive nitrogen balance** occurs when nitrogen intake exceeds nitrogen excretion. It is observed in situations in which tissue growth occurs, for example, in childhood, pregnancy, or during recovery from an emaciating illness. **Negative nitrogen balance** occurs when nitrogen losses are greater than nitrogen intake. It is associated with inadequate dietary protein; lack of an essential amino acid; or during physiologic stresses such as trauma, burns, illness, or surgery. **Kwashiorkor** occurs when protein deprivation is relatively greater than the reduction in total calories. It is characterized by edema. Marasmus occurs when calorie deprivation is relatively greater than the reduction in protein. Both are extreme forms of protein-energy malnutrition (PEM).

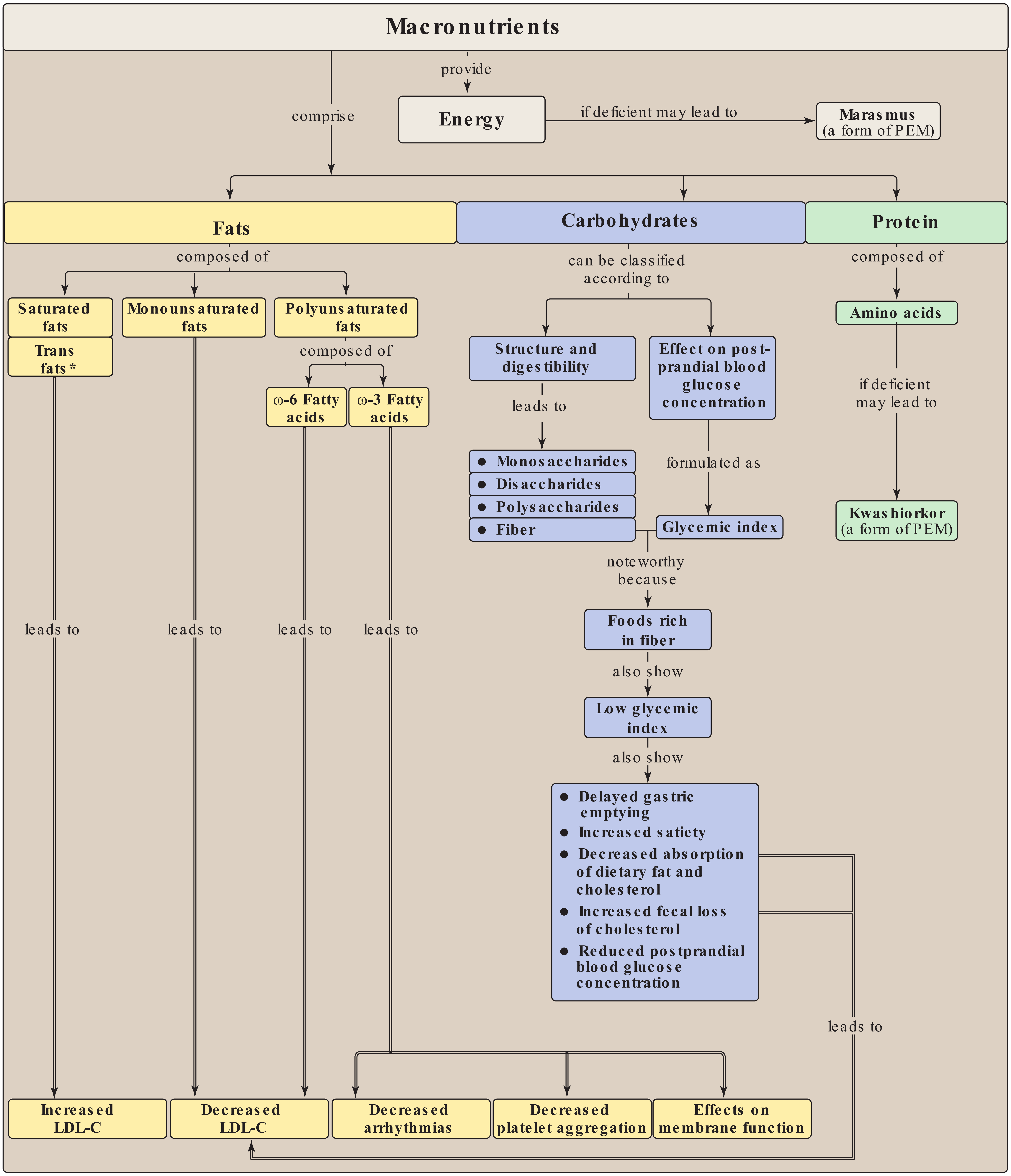

Macronutrients
provide
Energy
if deficient may lead to
Marasmus
(a form of PEM)
comprise
Fats
Carbohydrates
Protein
composed of
Saturated fats
Monounsaturated fats
Polyunsaturated fats
Trans fats*
composed of
ω-6 Fatty acids
ω-3 Fatty acids
can be classified according to
Structure and digestibility
Effect on post-prandial blood glucose concentration
leads to
● Monosaccharides
● Disaccharides
● Polysaccharides
● Fiber
formulated as
Glycemic index
noteworthy because
Foods rich in fiber
also show
Low glycemic index
also show
● Delayed gastric emptying
● Increased satiety
● Decreased absorption of dietary fat and cholesterol
● Increased fecal loss of cholesterol
● Reduced postprandial blood glucose concentration
composed of
Amino acids
if deficient may lead to
Kwashiorkor
(a form of PEM)
leads to
leads to
leads to
leads to
leads to
Increased LDL-C
Decreased LDL-C
Decreased arrhythmias
Decreased platelet aggregation
Effects on membrane function

Figure 27.23
Key concept map for the macronutrients. *Note: Trans fatty acids are chemically classified as unsaturated. PEM = protein energy malnutrition; LDL = low-density lipoprotein; C = cholesterol.

Vitamins

28

I. OVERVIEW

Vitamins are chemically unrelated organic compounds that cannot be synthesized in adequate quantities by humans and, therefore, must be supplied by the diet. Nine vitamins (folic acid, cobalamin, ascorbic acid, pyridoxine, thiamine, niacin, riboflavin, biotin, and pantothenic acid) are classified as water soluble. Because they are readily excreted in the urine, toxicity is rare. However, deficiencies can occur quickly. Four vitamins (A, D, K, and E) are termed fat soluble (Figure 28.1). They are released, absorbed, and transported (in chylomicrons) with dietary fat. They are not readily excreted, and significant quantities are stored in the liver and adipose tissue. In fact, consumption of vitamins A and D in excess of the Dietary Reference Intakes can lead to accumulation of toxic quantities of these compounds. Vitamins are required to perform specific cellular functions. For example, many of the water-soluble vitamins are precursors of coenzymes for the enzymes of intermediary metabolism. In contrast to the water-soluble vitamins, only one fat-soluble vitamin (vitamin K) has a coenzyme function.

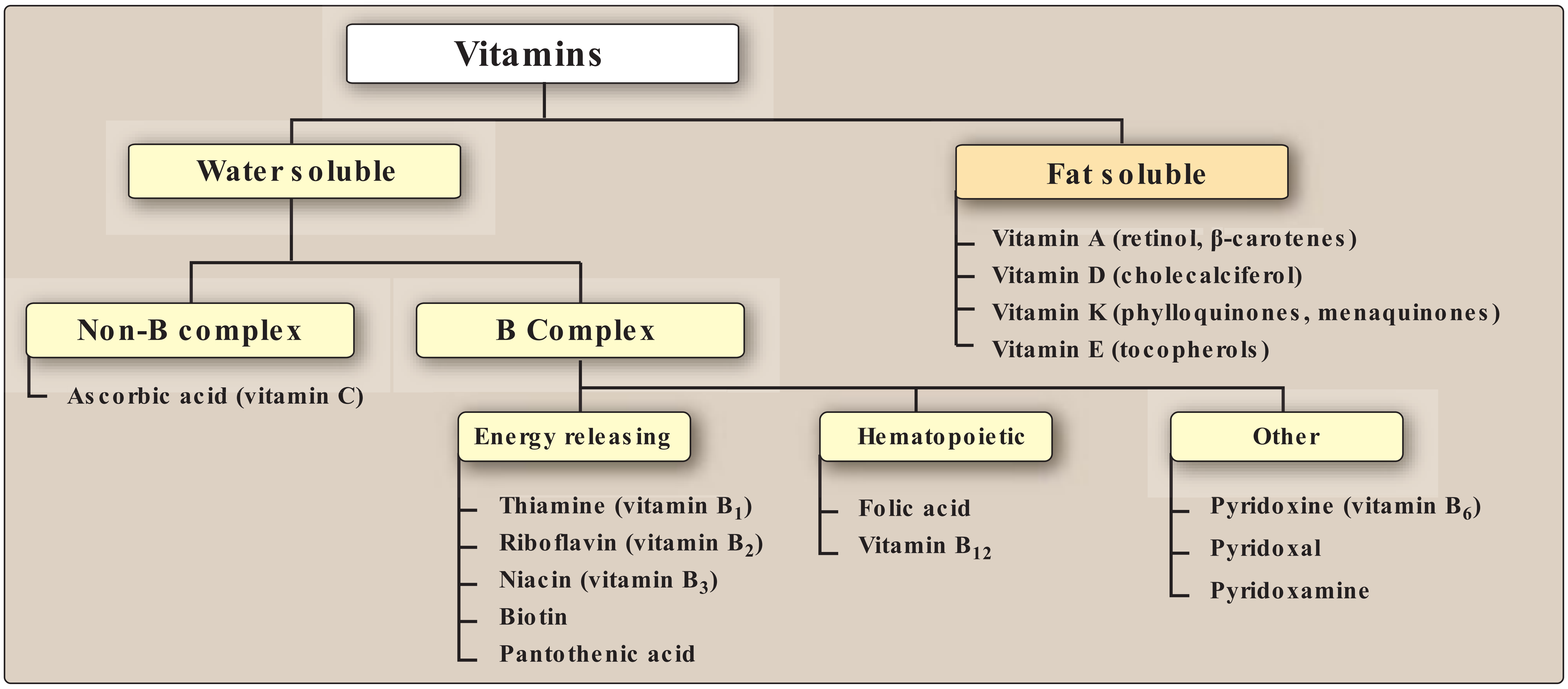

Vitamins
Water soluble
Fat soluble
Vitamin A (retinol, β-carotenes)
Vitamin D (cholecalciferol)
Vitamin K (phylloquinones, menaquinones)
Vitamin E (tocopherols)
Non-B complex
B Complex
Ascorbic acid (vitamin C)
Energy releasing
Hematopoietic
Other
Thiamine (vitamin B1)
Riboflavin (vitamin B2)
Niacin (vitamin B3)
Biotin
Pantothenic acid
Folic acid
Vitamin B12
Pyridoxine (vitamin B6)
Pyridoxal
Pyridoxamine

Figure 28.1
Classification of the vitamins.

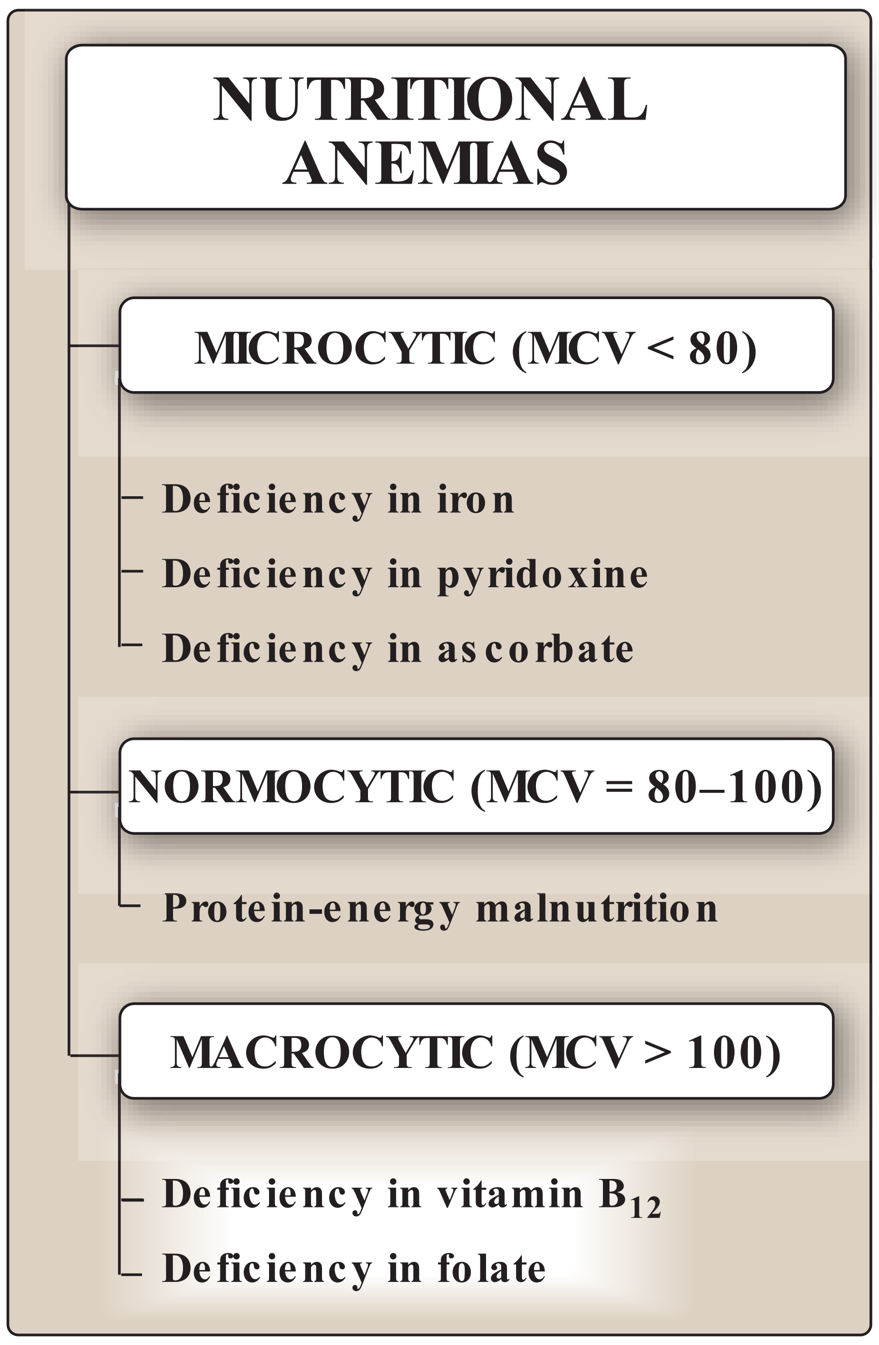

NUTRITIONAL ANEMIAS
MICROCYTIC (MCV < 80)
Deficiency in iron
Deficiency in pyridoxine
Deficiency in ascorbate
NORMOCYTIC (MCV = 80–100)
Protein-energy malnutrition
MACROCYTIC (MCV > 100)
Deficiency in vitamin B12
Deficiency in folate

Figure 28.2
Classification of nutritional anemias by cell size. The normal mean corpuscular volume (MCV) for people older than age 18 is between 80 and 100 µm^3. [Note: Microcytic anemia is also seen with lead poisoning.]

II. FOLIC ACID

Folic acid (or folate), which plays a key role in one-carbon metabolism, is essential for the biosynthesis of several compounds. Folic acid deficiency is probably the most common vitamin deficiency in the United States, particularly among pregnant women and alcoholics. [Note: Leafy, dark green vegetables are a good source of folic acid.]

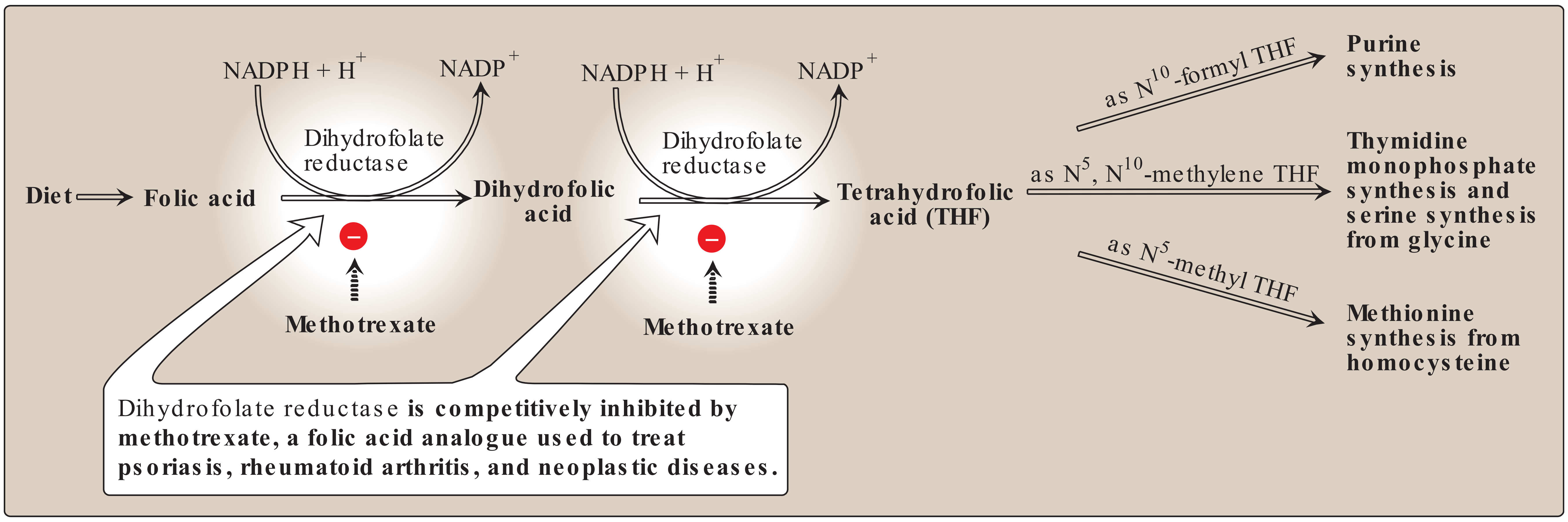
A. Function of folic acid

Tetrahydrofolate (THF), the reduced, coenzyme form of folate, receives one-carbon fragments from donors such as serine, glycine, and histidine and transfers them to intermediates in the synthesis of amino acids, purines, and thymidine monophosphate (TMP), a pyrimidine nucleotide found in DNA (Figure 28.3).

B. Nutritional anemias

Anemia is a condition in which the blood has a lower than normal concentration of hemoglobin, which results in a reduced ability to transport oxygen. Nutritional anemias (that is, those caused by inadequate intake of one or more essential nutrients) can be classified according to the size of the red blood cells (RBCs) or mean corpuscular volume (MCV) observed in the individual (Figure 28.2). Microcytic anemia (MCV below normal), caused by lack of iron, is the most common form of nutritional anemia. The second major category of nutritional anemia, macrocytic (MCV above normal), results from a deficiency in folic acid, or vitamin B_{12}. [Note: These macrocytic anemias are commonly called megaloblastic because a deficiency of either vitamin (or both) causes accumulation of large, immature RBC precursors, known as megaloblasts, in the bone marrow and the blood.]

1. **Folate and anemia:** Inadequate serum levels of folate can be caused by increased demand (for example, pregnancy and lactation), poor absorption caused by pathology of the small intestine, alcoholism, or treatment with drugs (for example, methotrexate) that are dihydrofolate reductase inhibitors (see Figure 28.3). A


NADPH + H$^+$
NADP$^+$
NADPH + H$^+$
NADP$^+$
Dihydrofolate reductase
Dihydrofolate reductase
Diet
Folic acid
Dihydrofolic acid
Tetrahydrofolic acid (THF)
Methotrexate
Methotrexate
as N^{10}-formyl THF
Purine synthesis
as N^5, N^{10}-methylene THF
Thymidine monophosphate synthesis and serine synthesis from glycine
as N^5-methyl THF
Methionine synthesis from homocysteine

Dihydrofolate reductase **is competitively inhibited by methotrexate, a folic acid analogue used to treat psoriasis, rheumatoid arthritis, and neoplastic diseases.**

Figure 28.3
Production and use of tetrahydrofolate. NADP(H) = nicotinamide adenine dinucleotide phosphate.

folate-free diet can cause a deficiency within a few weeks. A primary result of folic acid deficiency is megaloblastic anemia (Figure 28.4), caused by diminished synthesis of purines and TMP, which leads to an inability of cells (including RBC precursors) to make DNA and, therefore, an inability to divide.

2. **Folate and neural tube defects in the fetus:** Spina bifida and anencephaly, the most common neural tube defects (NTDs), affect approximately 3,000 pregnancies in the United State annually. Folic acid supplementation before conception and during the first trimester has been shown to significantly reduce NTDs. Therefore, all women of childbearing age are advised to consume 0.4 mg/day of folic acid to reduce the risk of having a pregnancy affected by NTDs and ten times that amount if a previous pregnancy was affected. Adequate folate nutrition must occur at the time of conception because critical folate-dependent development occurs in the first weeks of fetal life, at a time when many women are not yet aware of their pregnancy. In 1998, the U.S. Food and Drug Administration authorized the addition of folic acid to enriched grain products, resulting in a dietary supplementation of about 0.1 mg/day. It is estimated that this supplementation allows approximately 50% of all reproductive-aged women to receive 0.4 mg of folate from all sources. [Note: High-dose folate supplementation can mask the symptoms of vitamin B_{12} deficiency (see blue box below) and is not recommended for most adults.]

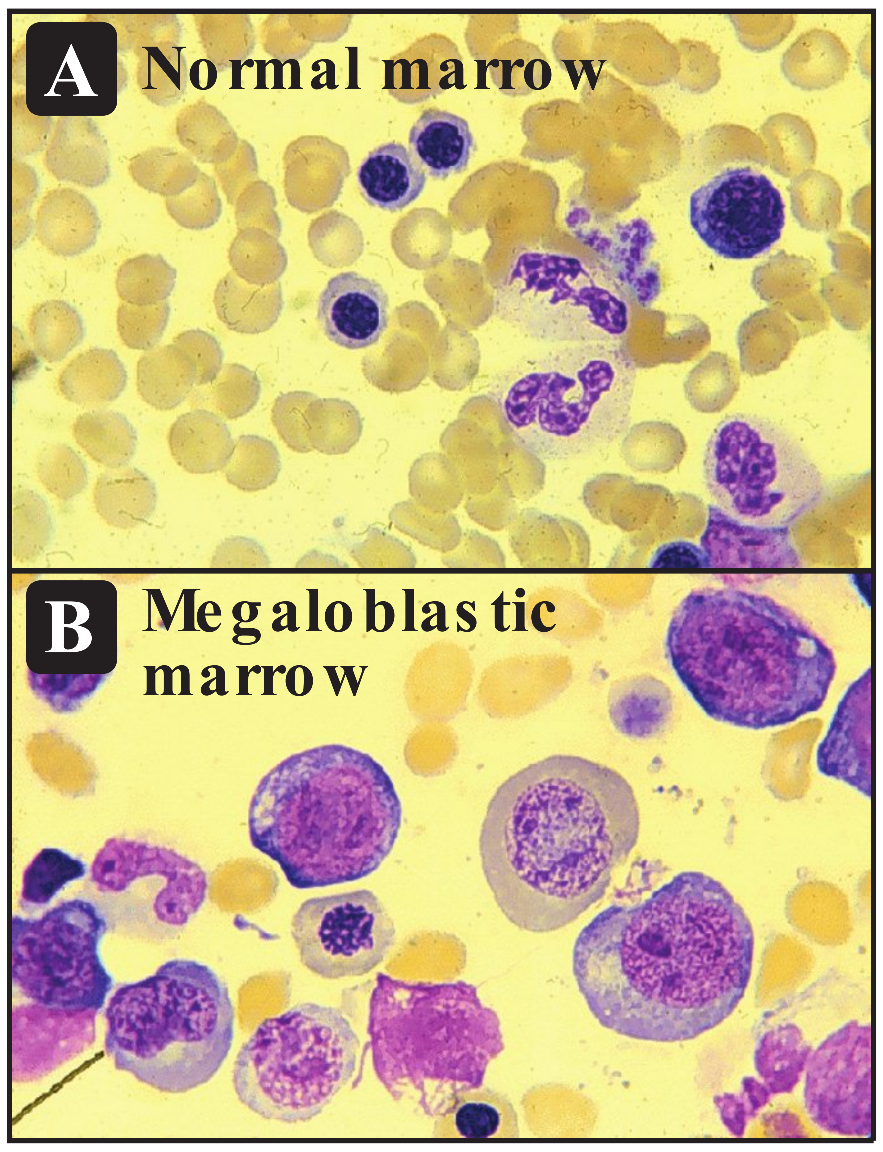


Figure 28.4
Bone marrow histology in normal and folate-deficient individuals.

III. COBALAMIN (VITAMIN B_{12})

Vitamin B_{12} is required in humans for two essential enzymatic reactions: the remethylation of homocysteine (Hcy) to methionine and the isomerization of methylmalonyl coenzyme A (CoA), which is produced during the degradation of some amino acids (isoleucine, valine, threonine, and methionine) and fatty acids (FAs) with odd numbers of carbon atoms (Figure 28.5). When cobalamin is deficient, unusual (branched) FAs accumulate and become incorporated into cell membranes, including those of the central nervous system (CNS). This may account for some of the neurologic manifestations of vitamin B_{12} deficiency. [Note: Folic acid (as N^5-methyl THF) is also required in the remethylation of Hcy. Therefore, deficiency of B_{12} or folate results in elevated Hcy levels.]

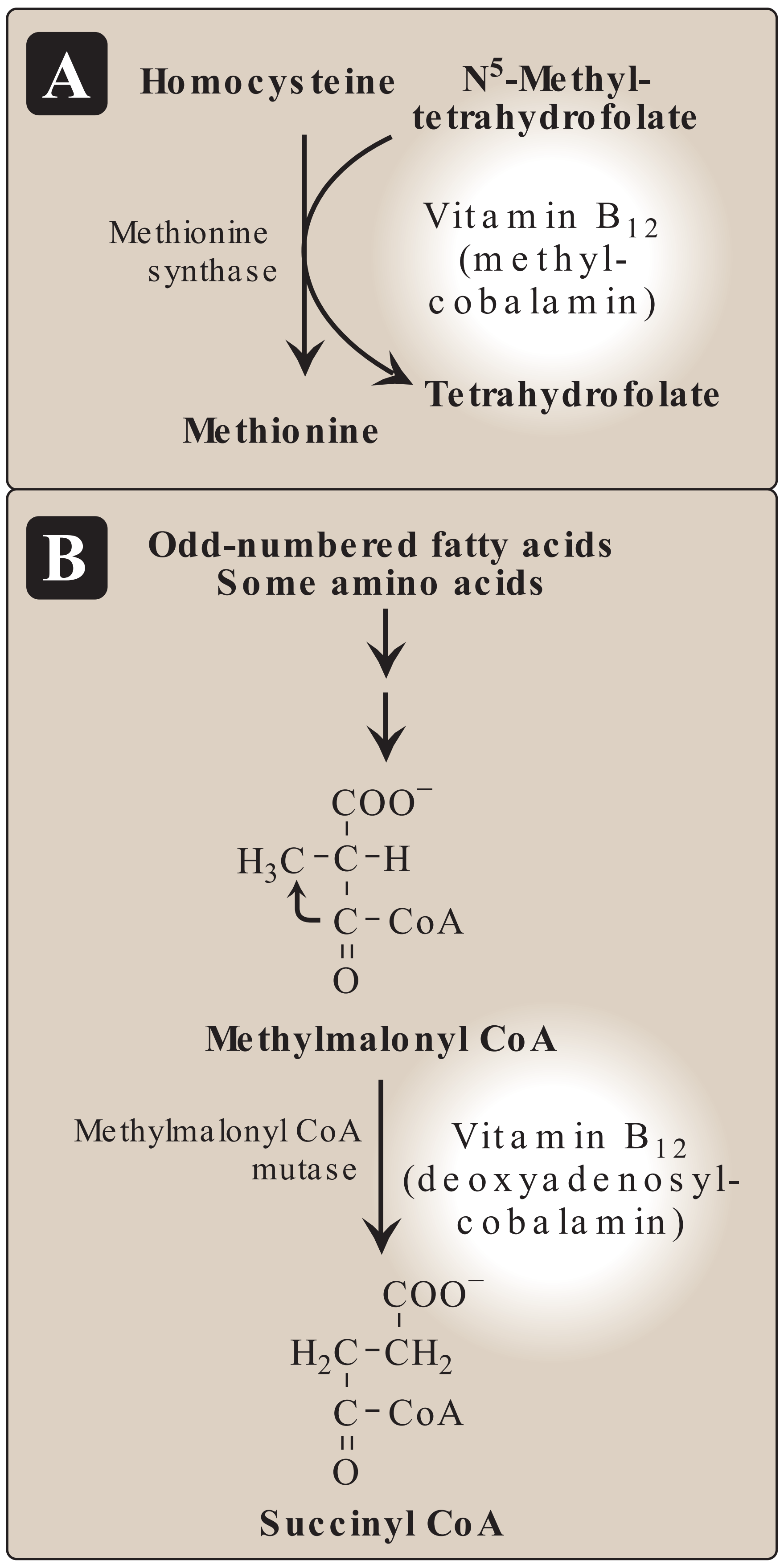


Figure 28.5
Reactions requiring coenzyme forms of vitamin B_{12}. CoA = coenzyme A.

A. Structure of cobalamin and its coenzyme forms

Cobalamin contains a corrin ring system that resembles the porphyrin ring of heme (see p. 280), and but differs in that two of the pyrrole rings are linked directly rather than through a methene bridge. Cobalt is held in the center of the corrin ring by four coordination bonds with the nitrogens of the pyrrole groups. The remaining coordination bonds of the cobalt are with the nitrogen of 5,6-dimethylbenzimidazole and with cyanide in commercial preparations of the vitamin in the form of cyanocobalamin (Figure 28.6). The physiologic coenzyme forms of cobalamin are 5'-deoxyadenosylcobalamin and methylcobalamin, in which cyanide is replaced with 5'-deoxyadenosine or a methyl group, respectively (see Figure 28.6).

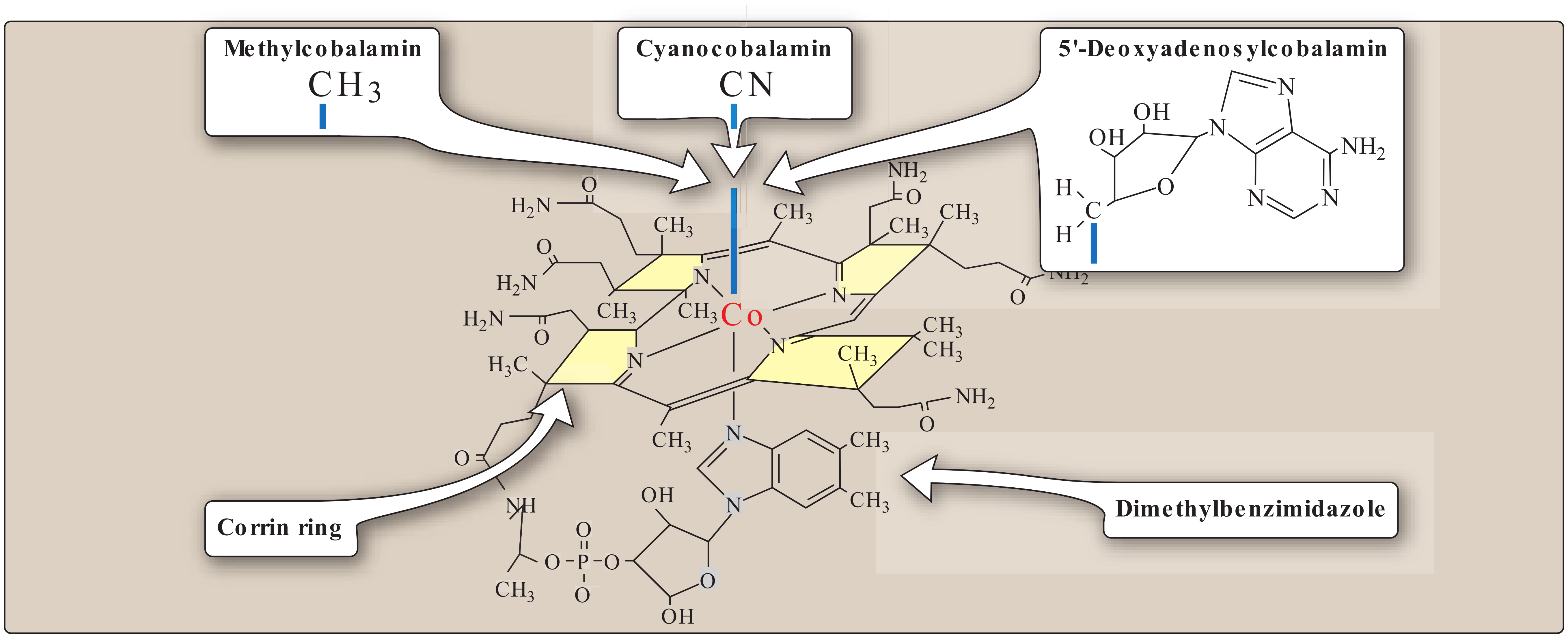


Figure 28.6
Structure of vitamin B_{12} (cyanocobalamin) and its coenzyme forms (methylcobalamin and 5'-deoxyadenosylcobalamin).

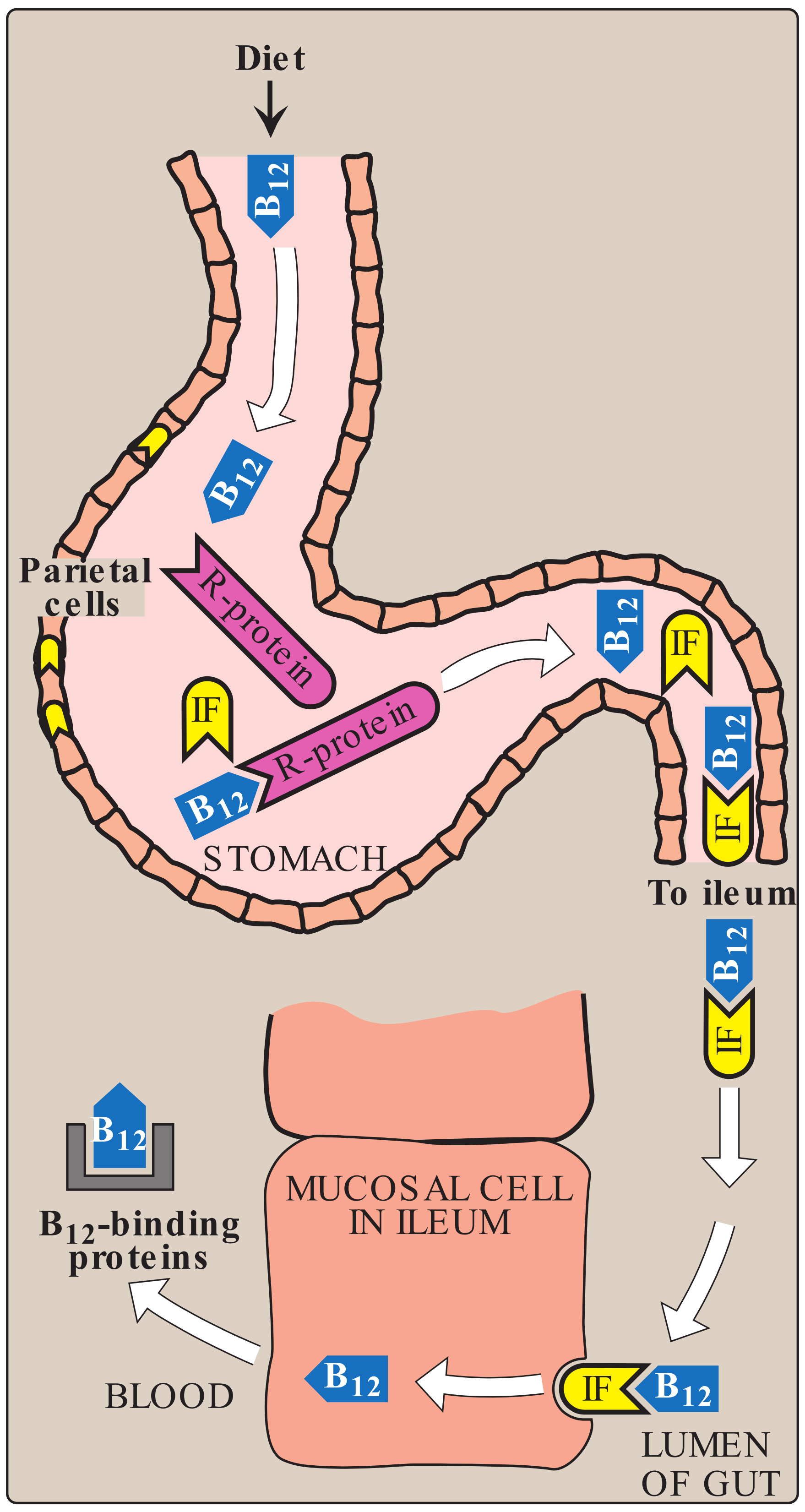


Figure 28.7
Absorption of vitamin B_{12}. IF = intrinsic factor.

B. Distribution of cobalamin

Vitamin B_{12} is synthesized only by microorganisms, and it is not present in plants. Animals obtain the vitamin preformed from their natural bacterial flora or by eating foods derived from other animals. Cobalamin is present in appreciable amounts in liver, red meat, fish, eggs, dairy products, and fortified cereals.

C. Folate trap hypothesis

The effects of cobalamin deficiency are most pronounced in rapidly dividing cells, such as the erythropoietic tissue of bone marrow and the mucosal cells of the intestine. Such tissues need both the N^5,N^{10}-methylene and N^{10}-formyl forms of THF for the synthesis of nucleotides required for DNA replication (see pp. 293 and 303). However, in vitamin B_{12} deficiency, the utilization of the N^5-methyl form of THF in the B_{12}-dependent methylation of homocysteine to methionine is impaired. Because the methylated form cannot be converted directly to other forms of THF, folate is trapped in the N^5-methyl form, which accumulates. The levels of the other forms decrease. Thus, cobalamin deficiency leads to a deficiency of the THF forms needed in purine and TMP synthesis, resulting in the symptoms of megaloblastic anemia.

D. Clinical indications for vitamin B_{12}

In contrast to other water-soluble vitamins, significant amounts (2–5 mg) of vitamin B_{12} are stored in the body. As a result, it may take several years for the clinical symptoms of B_{12} deficiency to develop as a result of decreased intake of the vitamin. [Note: Deficiency happens much more quickly if absorption is impaired (see below).] B_{12} deficiency can be determined by the level of methylmalonic acid in blood, which is elevated in individuals with low intake or decreased absorption of the vitamin.

1. **Pernicious anemia:** Vitamin B_{12} deficiency is most commonly seen in patients who fail to absorb the vitamin from the intestine. B_{12}

is released from food in the acidic environment of the stomach. [Note: Malabsorption of cobalamin in the elderly is most often due to reduced secretion of gastric acid (achlorhydria).] Free B_{12} then binds a glycoprotein (R-protein), and the complex moves into the intestine. B_{12} is released from the R-protein by pancreatic enzymes and binds another glycoprotein, intrinsic factor (IF). The cobalamin–IF complex travels through the intestine and binds to specific receptors on the surface of mucosal cells in the ileum. The cobalamin is transported into the mucosal cell and, subsequently, into the general circulation, where it is carried by its binding protein (transcobalamin). B_{12} is taken up and stored in the liver, primarily. It is released into bile and efficiently reabsorbed in the ileum. Severe malabsorption of vitamin B_{12} leads to pernicious anemia. This disease is most commonly a result of an autoimmune destruction of the gastric parietal cells that are responsible for the synthesis of IF (lack of IF prevents B_{12} absorption). [Note: Patients who have had a partial or total gastrectomy become IF deficient and, therefore, B_{12} deficient.] Individuals with cobalamin deficiency are usually anemic, and they show neuropsychiatric symptoms later, as the disease develops. The CNS effects are irreversible and occur by mechanisms that appear to be different from those described for megaloblastic anemia. Pernicious anemia requires life-long treatment with either high-dose oral B_{12} or intramuscular injection of cyanocobalamin. [Note: Supplementation works even in the absence of IF because approximately 1% of B_{12} uptake is by IF-independent diffusion.]

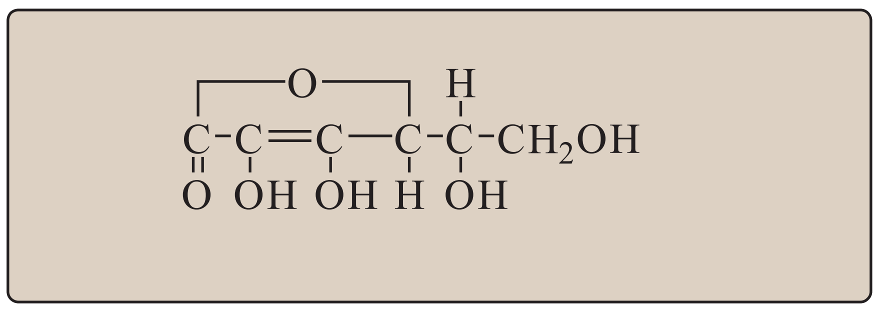


Figure 28.8
Structure of ascorbic acid.

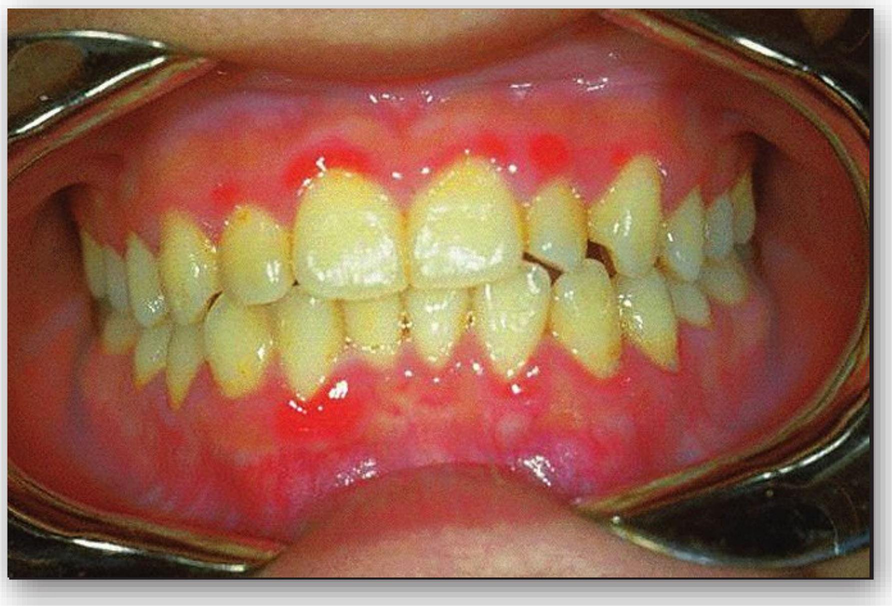


Figure 28.9
Hemorrhage and swollen gums of a patient with scurvy.

> Folic acid supplementation can partially reverse the hematologic abnormalities of B_{12} deficiency and, therefore, can mask a cobalamin deficiency. Thus, to prevent the CNS effects of B_{12} deficiency, therapy for megaloblastic anemia is initiated with both vitamin B_{12} and folic acid until the cause of the anemia can be determined.

IV. ASCORBIC ACID (VITAMIN C)

The active form of vitamin C is ascorbic acid (Figure 28.8). The main function of ascorbate is as a reducing agent in several different reactions. Vitamin C has a well-documented role as a coenzyme in hydroxylation reactions (for example, hydroxylation of prolyl and lysyl residues of collagen; see p. 47). Vitamin C is, therefore, required for the maintenance of normal connective tissue as well as for wound healing. Vitamin C also reduces ferric iron to the ferrous form, thereby facilitating the absorption of dietary iron from the intestine.

A. Deficiency of ascorbic acid

A deficiency of ascorbic acid results in scurvy, a disease characterized by sore and spongy gums, loose teeth, fragile blood vessels, swollen joints, fatigue, and a microcytic anemia caused by decreased absorption of iron (Figure 28.9). Many of the deficiency symptoms can be explained by a deficiency in the hydroxylation of collagen, resulting in defective connective tissue.

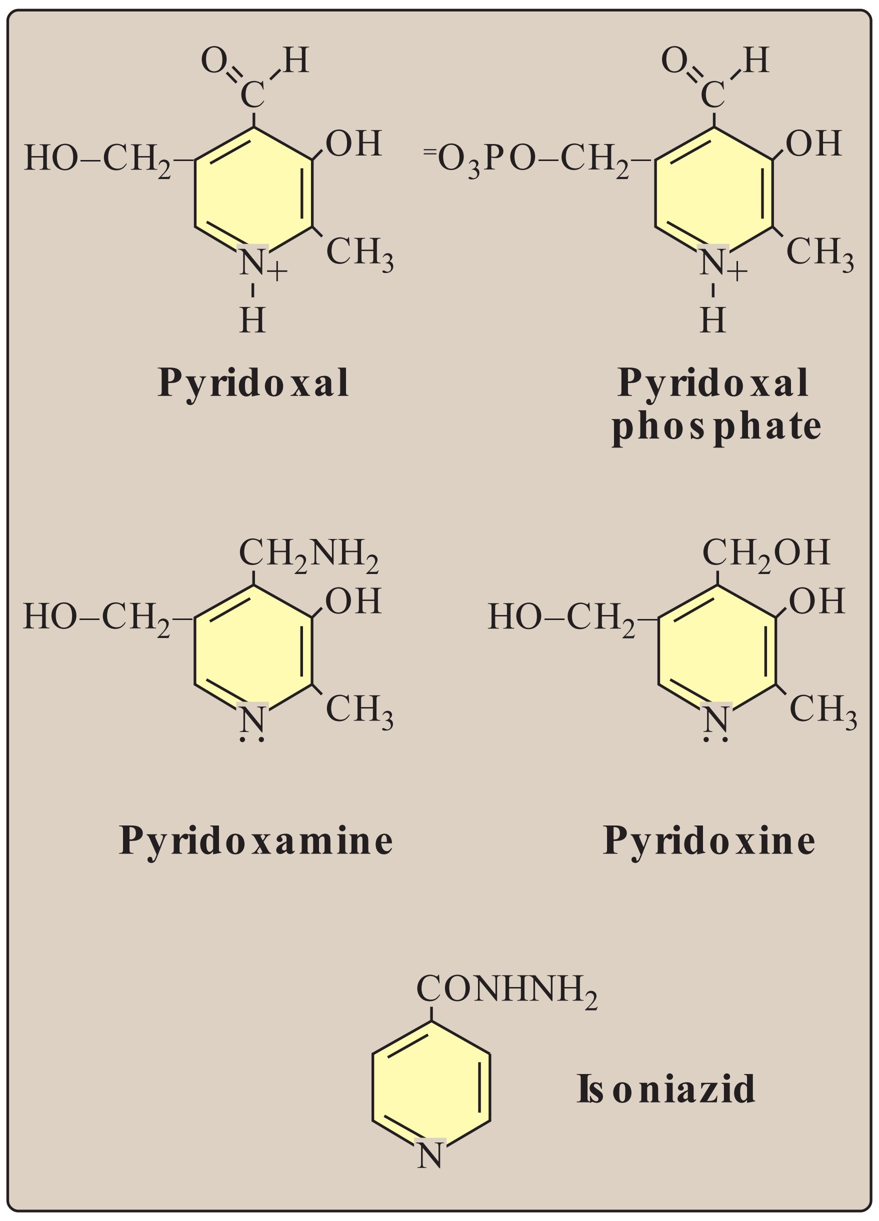


Figure 28.10
Structures of vitamin B_6 and the antituberculosis drug isoniazid.

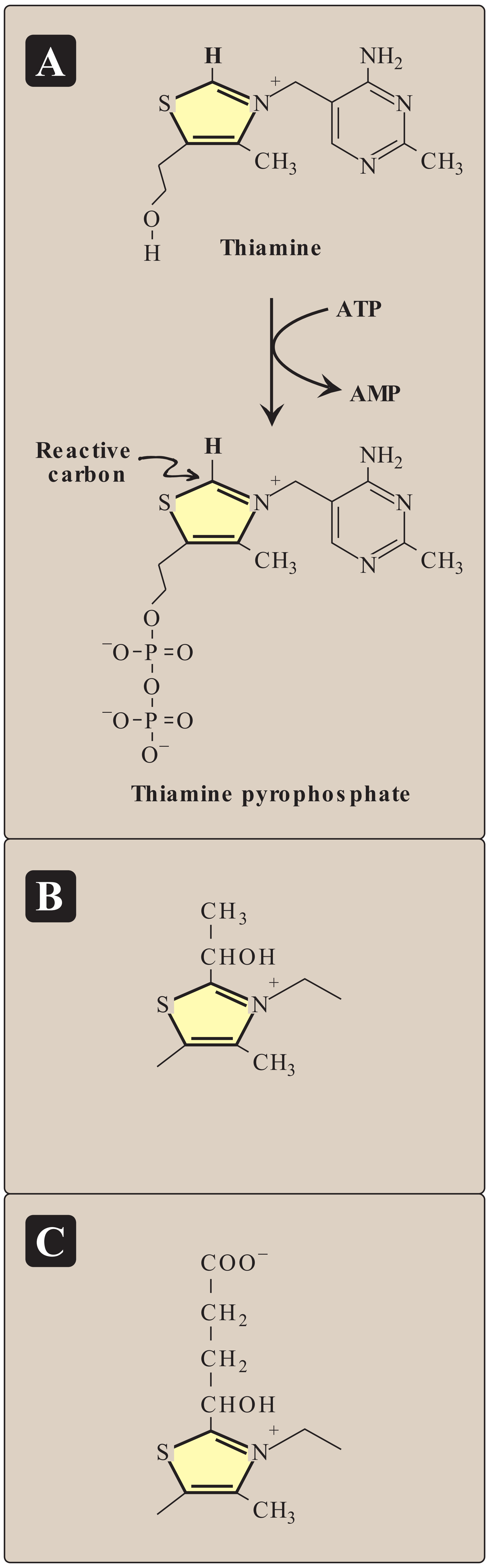


Figure 28.11
A. Structure of thiamine and its coenzyme form, thiamine pyrophosphate. B. Structure of intermediate formed in the reaction catalyzed by pyruvate dehydrogenase. C. Structure of intermediate formed in the reaction catalyzed by α-ketoglutarate dehydrogenase. AMP = adenosine monophosphate.

B. Prevention of chronic disease

Vitamin C is one of a group of nutrients that includes vitamin E (see p. 391) and β-carotene (see p. 382), which are known as antioxidants. [Note: Ascorbate regenerates the functional, reduced form of vitamin E.] Consumption of diets rich in these compounds is associated with a decreased incidence of some chronic diseases, such as coronary heart disease and certain cancers. However, clinical trials involving supplementation with the isolated antioxidants have failed to demonstrate any convincing beneficial effects.

V. PYRIDOXINE (VITAMIN B_6)

Vitamin B_6 is a collective term for pyridoxine, pyridoxal, and pyridoxamine, all derivatives of pyridine. They differ only in the nature of the functional group attached to the ring (Figure 28.10). Pyridoxine occurs primarily in plants, whereas pyridoxal and pyridoxamine are found in foods obtained from animals. All three compounds can serve as precursors of the biologically active coenzyme, pyridoxal phosphate (PLP). PLP functions as a coenzyme for a large number of enzymes, particularly those that catalyze reactions involving amino acids, for example, in the synthesis of cysteine from Hcy (see p. 264). [Note: PLP is also required by glycogen phosphorylase (see p. 128).]

Reaction type	Example
Transamination	Oxaloacetate + glutamate ⇄ aspartate + α-ketoglutarate
Deamination	Serine → pyruvate + NH_3
Decarboxylation	Histidine → histamine + CO_2
Condensation	Glycine + succinyl CoA → δ-aminolevulinic acid

A. Clinical indications for pyridoxine

Isoniazid, a drug commonly used to treat tuberculosis, can induce a vitamin B_6 deficiency by forming an inactive derivative with PLP. Dietary supplementation with B_6 is, thus, an adjunct to isoniazid treatment. Otherwise, dietary deficiencies in pyridoxine are rare but have been observed in newborn infants fed formulas low in B_6, in women taking oral contraceptives, and in alcoholics.

B. Toxicity of pyridoxine

Pyridoxine is the only water-soluble vitamin with significant toxicity. Neurologic symptoms (sensory neuropathy) occur at intakes above 500 mg/day, an amount nearly 400 times the Recommended Dietary Allowance (RDA) and over 5 times the Tolerable Upper Limit (UL). Substantial improvement, but not complete recovery, occurs when the vitamin is discontinued.

VI. THIAMINE (VITAMIN B_1)

Thiamine pyrophosphate (TPP) is the biologically active form of the vitamin, formed by the transfer of a pyrophosphate group from adenosine triphosphate (ATP) to thiamine (Figure 28.11). TPP serves as a coenzyme in the formation or degradation of α-ketols by transketolase (Figure 28.12A), and in the oxidative decarboxylation of α-keto acids (Figure 28.12B).

A. Clinical indications for thiamine

The oxidative decarboxylation of pyruvate and α-ketoglutarate, which plays a key role in energy metabolism of most cells, is particularly important in tissues of the CNS. In thiamine deficiency, the activity of these two dehydrogenase-catalyzed reactions is decreased, resulting in decreased production of ATP and, therefore, impaired cellular function. TPP is also required by branched-chain *α*-keto acid dehydrogenase of muscle. [Note: It is the decarboxylase of each of these *α*-keto acid dehydrogenase multienzyme complexes that requires TPP.] Thiamine deficiency is diagnosed by an increase in erythrocyte transketolase activity observed on addition of TPP.]

1. **Beriberi:** This is a severe thiamine-deficiency syndrome found in areas where polished rice is the major component of the diet. Adult beriberi is classified as dry (characterized by peripheral neurologic deficits) or wet (characterized by edema due to cardiac dysfunction). Infantile beriberi is seen in nursing infants whose mothers are deficient in thiamine.

2. **Wernicke-Korsakoff syndrome:** In the United States, thiamine deficiency, which is seen primarily in association with chronic alcoholism, is due to dietary insufficiency or impaired intestinal absorption of the vitamin. Some alcoholics develop Wernicke-Korsakoff syndrome, a thiamine deficiency state characterized by confusion, ataxia, and a rhythmic to-and-fro motion of the eyeballs (nystagmus) with Wernicke encephalopathy as well as memory problems and hallucinations with Korsakoff dementia. The syndrome is treatable with thiamine supplementation, but recovery of memory is typically incomplete.

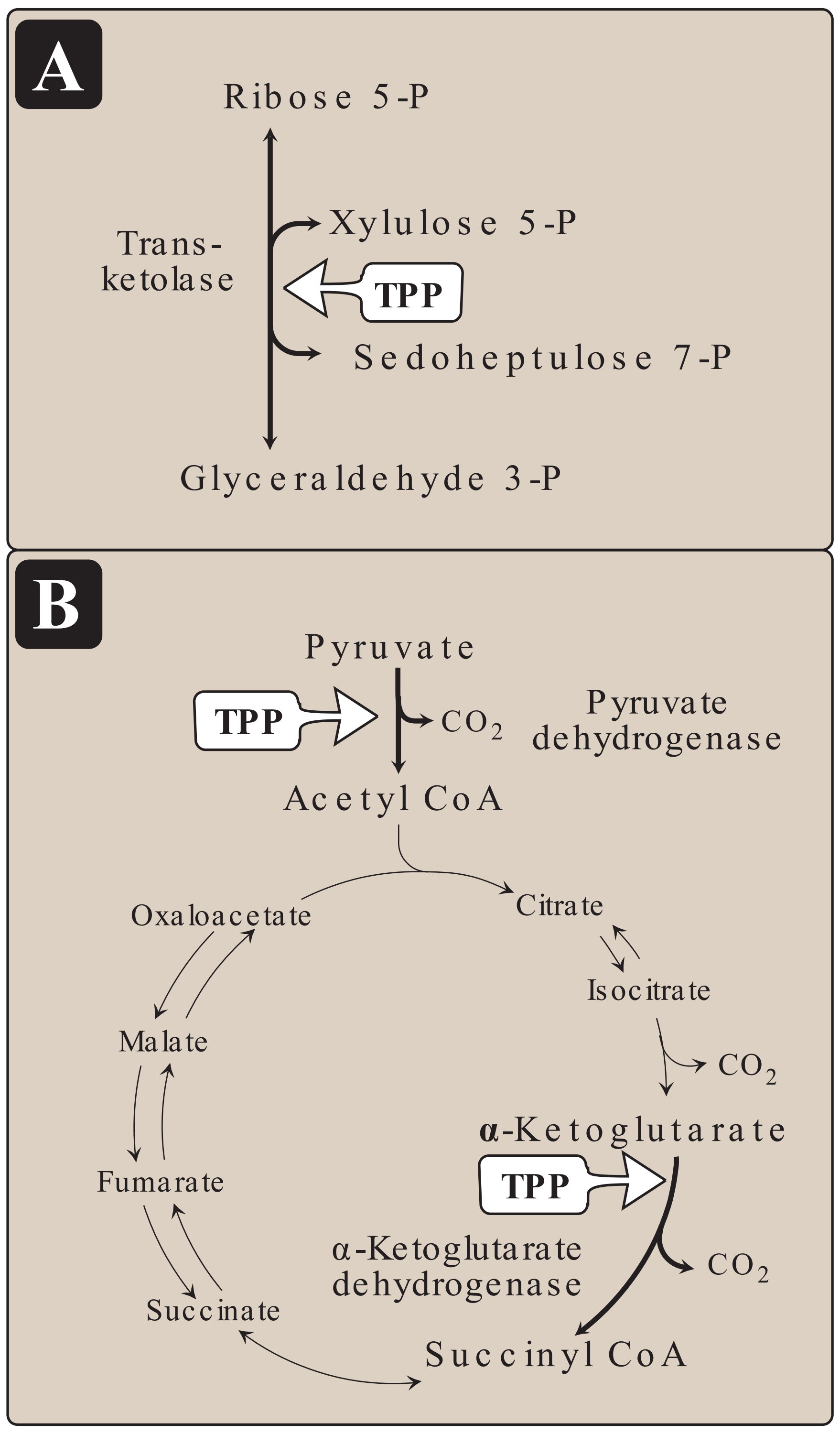

A
Ribose 5-P
Trans-
ketolase
Xylulose 5-P
TPP
Sedoheptulose 7-P
Glyceraldehyde 3-P
B
Pyruvate
TPP
CO2
Pyruvate
dehydrogenase
Acetyl CoA
Oxaloacetate
Citrate
Isocitrate
CO2
Malate
α-Ketoglutarate
TPP
Fumarate
α-Ketoglutarate
dehydrogenase
CO2
Succinate
Succinyl CoA

Figure 28.12
Reactions that use thiamine pyrophosphate (TPP) as coenzyme. A. Transketolase. B. Pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. Note that TPP is also used by branched-chain α-keto acid dehydrogenase. P = phosphate; CoA = coenzyme A.

VII. NIACIN

Niacin, or nicotinic acid, is a substituted pyridine derivative. The biologically active coenzyme forms are nicotinamide adenine dinucleotide

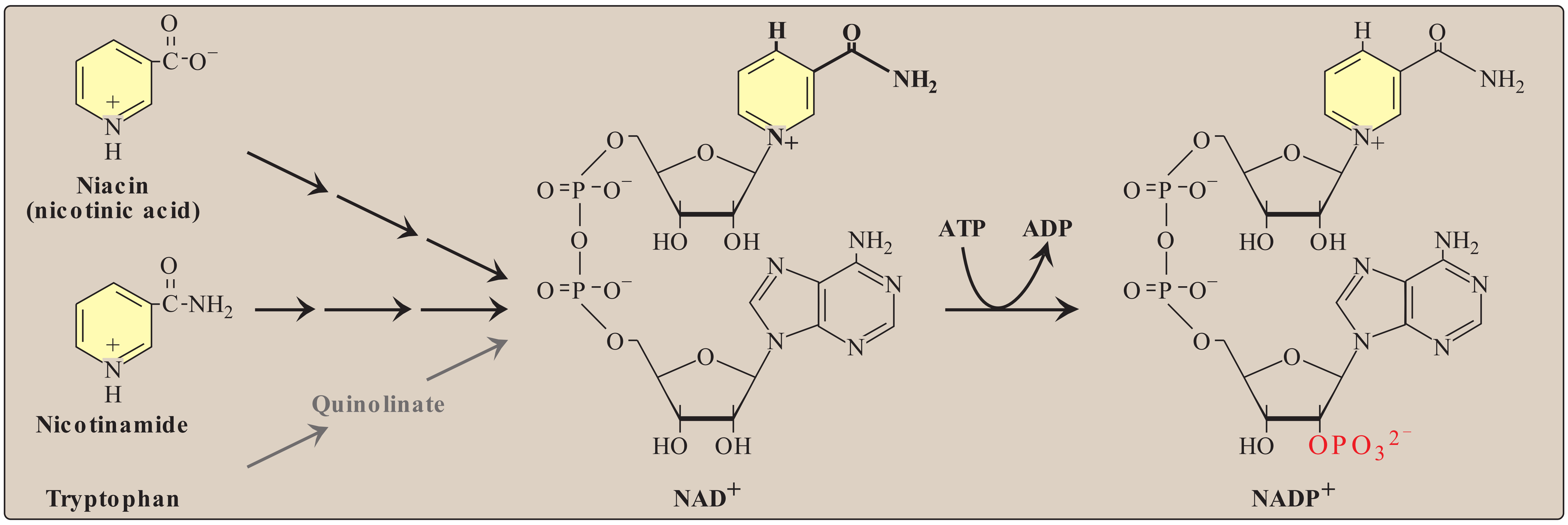

Niacin
(nicotinic acid)
Nicotinamide
Quinolinate
Tryptophan
ATP
ADP
NAD+
NADP+
OPO3 2−
NH2
HO OH

Figure 28.13
Structure and biosynthesis of oxidized nicotinamide adenine dinucleotide (NAD$^+$) and nicotinamide adenine dinucleotide phosphate (NADP$^+$). ADP = adenosine diphosphate.

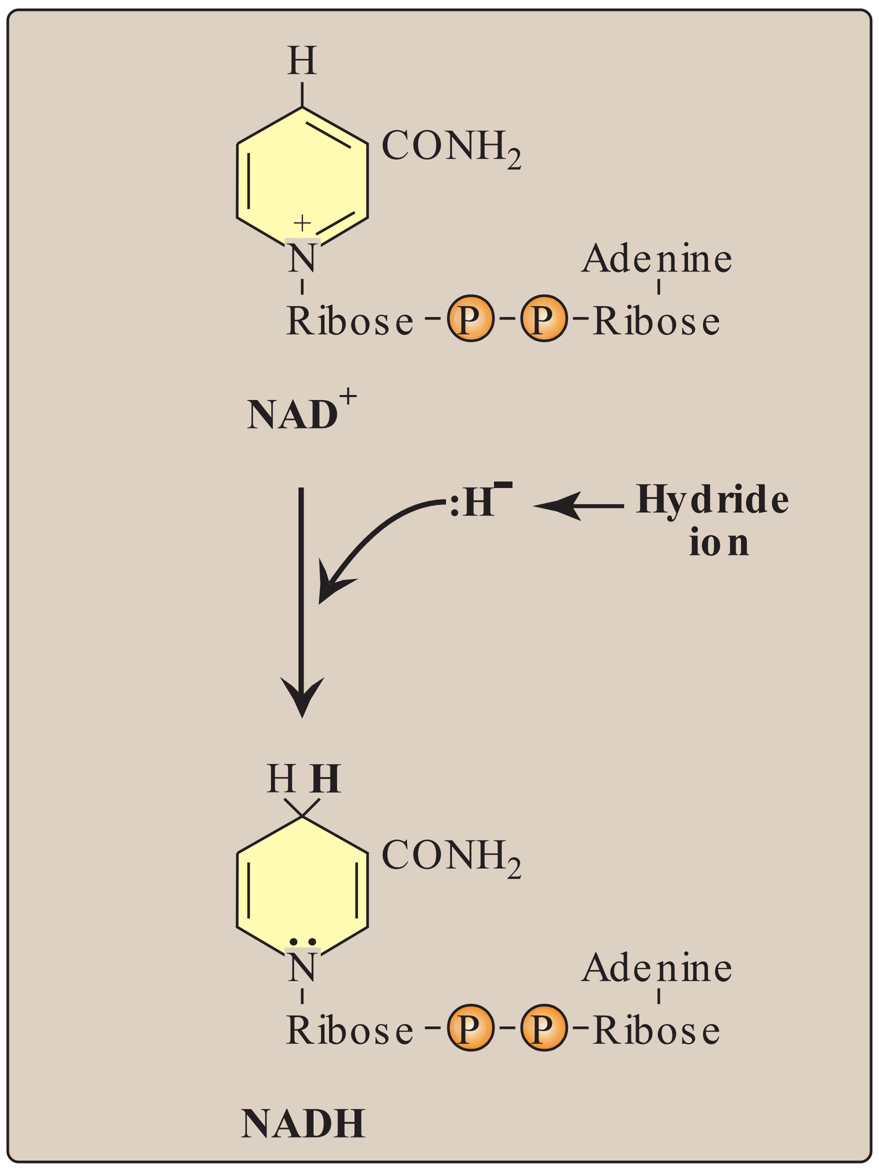


Figure 28.14
Reduction of oxidized nicotinamide adenine dinucleotide (NAD$^+$) to NADH. Ⓟ = phosphate.

(NAD$^+$) and its phosphorylated derivative, nicotinamide adenine dinucleotide phosphate (NADP$^+$) as shown in Figure 28.13. Nicotinamide, a derivative of nicotinic acid that contains an amide instead of a carboxyl group, also occurs in the diet. Nicotinamide is readily deaminated in the body and, therefore, is nutritionally equivalent to nicotinic acid. NAD$^+$ and NADP$^+$ serve as coenzymes in oxidation-reduction reactions in which the coenzyme undergoes reduction of the pyridine ring by accepting a hydride ion (hydrogen atom plus one electron) as shown in Figure 28.14. The reduced forms of NAD$^+$ and NADP$^+$ are NADH and NADPH, respectively. [Note: A metabolite of tryptophan, quinolinate, can be converted to NAD(P). In comparison, 60 mg of tryptophan = 1 mg of niacin.]

A. Distribution of niacin

Niacin is found in unrefined and enriched grains and cereal; milk; and lean meats, especially liver.

B. Clinical indications for niacin

1. **Deficiency of niacin:** A deficiency of niacin causes pellagra, a disease involving the skin, gastrointestinal tract, and CNS. The symptoms of pellagra progress through the three Ds: dermatitis; diarrhea; dementia; and, if untreated, death. Hartnup disorder, characterized by defective absorption of tryptophan, can result in pellagra-like symptoms. [Note: Corn is low in both niacin and tryptophan. Corn-based diets can cause pellagra.]

2. **Treatment of hyperlipidemia:** Niacin at doses of 1.5 g/day, or 100 times the RDA, strongly inhibits lipolysis in adipose tissue, the primary producer of circulating free fatty acids (FFAs). The liver normally uses these circulating FFAs as a major precursor for triacylglycerol (TAG) synthesis. Thus, niacin causes a decrease in liver TAG synthesis, which is required for very-low-density lipoprotein ([VLDL] see p. 231) production. Low-density lipoprotein (LDL, the cholesterol-rich lipoprotein) is derived from VLDL in the plasma. Thus, both plasma TAG (in VLDL) and cholesterol (in LDL) are lowered. Therefore, niacin is particularly useful in the treatment of type IIb hyperlipoproteinemia, in which both VLDL and LDL are elevated. The high doses of niacin required can cause acute, prostaglandin-mediated flushing. Aspirin can reduce this side effect by inhibiting prostaglandin synthesis (see p. 214). [Note: Niacin raises high-density lipoprotein levels.]

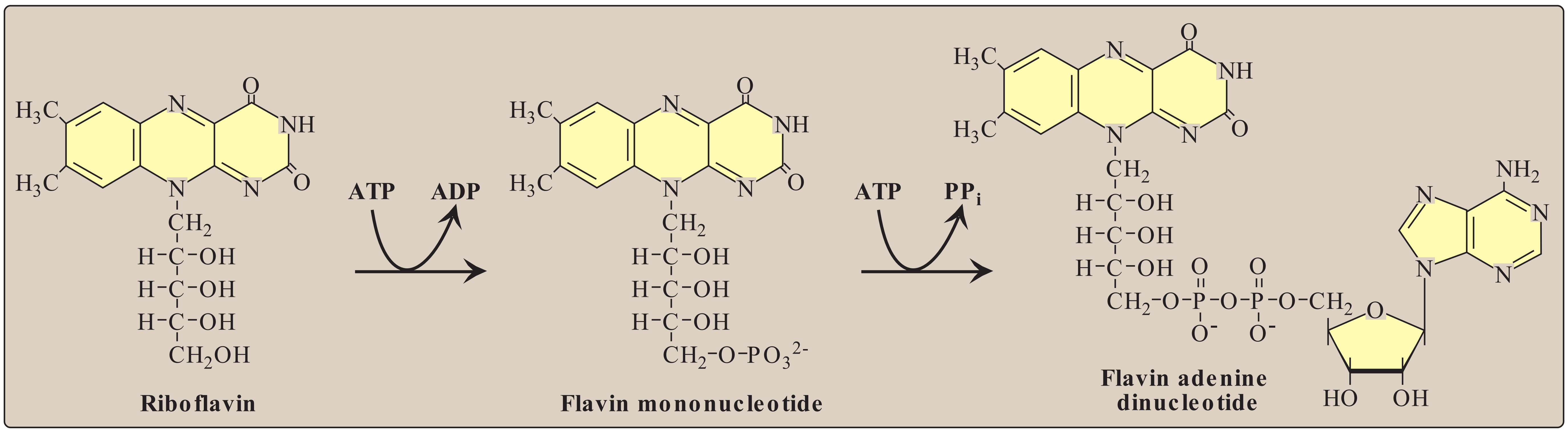


Figure 28.15
Structure and biosynthesis of the oxidized forms of flavin mononucleotide and flavin adenine dinucleotide. ADP = adenosine diphosphate; PP$_i$ = pyrophosphate.

VIII. RIBOFLAVIN (VITAMIN B$_2$)

The two biologically active forms of B$_2$ are flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), formed by the transfer of an adenosine monophosphate moiety from ATP to FMN (Figure 28.15). FMN and FAD are each capable of reversibly accepting two hydrogen atoms, forming FMNH$_2$ or FADH$_2$. FMN and FAD are bound tightly, sometimes covalently, to flavoenzymes (for example, NADH dehydrogenase [FMN] and succinate dehydrogenase [FAD]) that catalyze the oxidation or reduction of a substrate. Riboflavin deficiency is not associated with a major human disease, although it frequently accompanies other vitamin deficiencies. Deficiency symptoms include dermatitis, cheilosis (fissuring at the corners of the mouth), and glossitis (the tongue appearing smooth and dark).

IX. BIOTIN

Biotin is a coenzyme in carboxylation reactions, in which it serves as a carrier of activated carbon dioxide (see Figure 10.3, p. 119, for the mechanism of biotin-dependent carboxylations). Biotin is covalently bound to the ε-amino group of lysine residues in biotin-dependent enzymes (Figure 28.16). Biotin deficiency does not occur naturally because the vitamin is widely distributed in food. Also, a large percentage of the biotin requirement in humans is supplied by intestinal bacteria. However, the addition of raw egg white to the diet as a source of protein induces symptoms of biotin deficiency, namely, dermatitis, glossitis, loss of appetite, and nausea. Raw egg white contains a glycoprotein, avidin, which tightly binds biotin and prevents its absorption from the intestine. With a normal diet, however, it has been estimated that 20 eggs/day would be required to induce a deficiency syndrome. Thus, inclusion of an occasional raw egg in the diet does not lead to biotin deficiency, although eating raw eggs is generally not recommended due to the possibility of salmonella infection.

> Multiple carboxylase deficiency results from a defect in the ability to add biotin to carboxylases during their synthesis or to remove it from carboxylases during their degradation. Treatment is biotin supplementation.

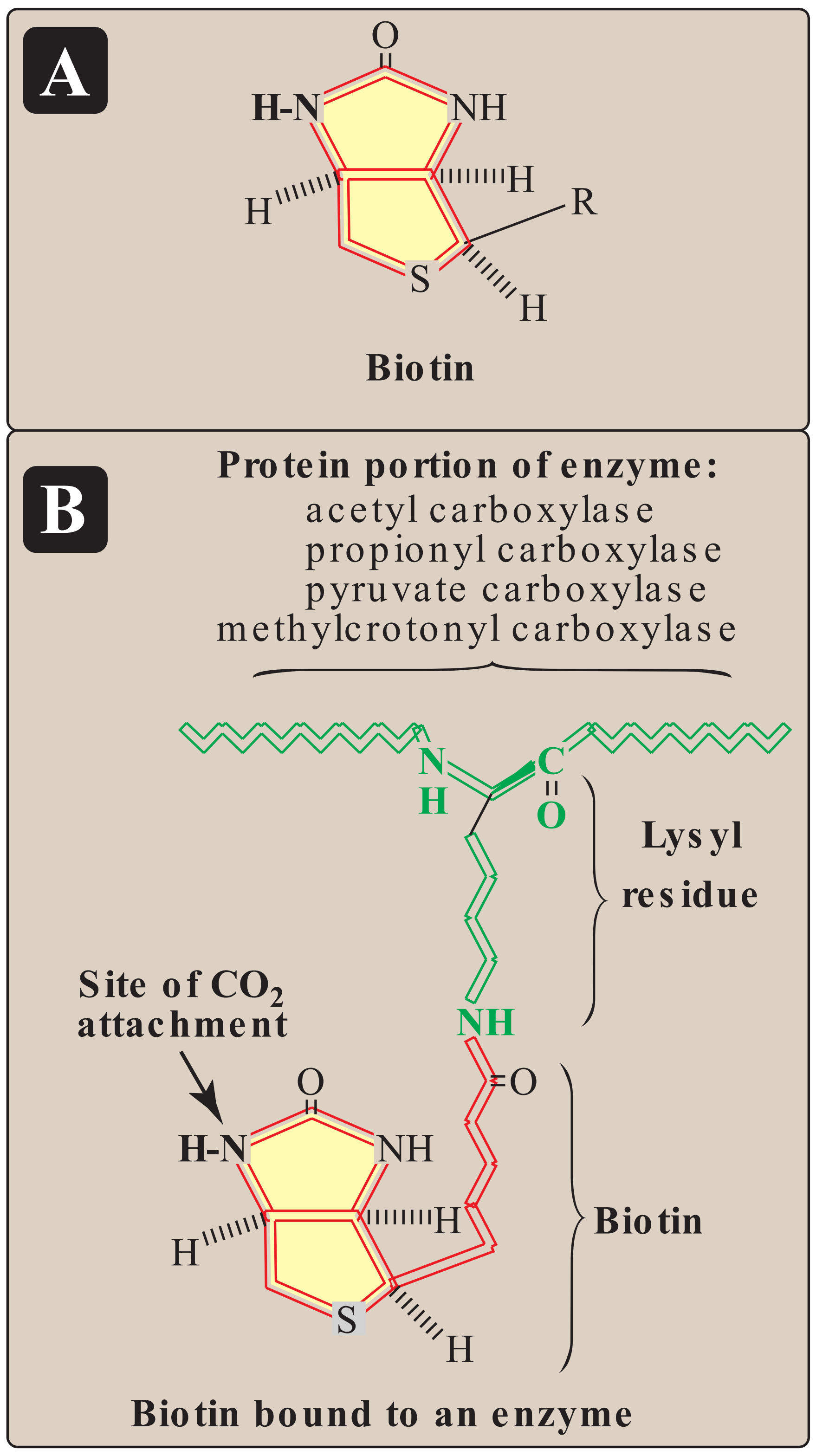

A
O
H-N
NH
H
H
R
S
H
Biotin
B
Protein portion of enzyme:
acetyl carboxylase
propionyl carboxylase
pyruvate carboxylase
methylcrotonyl carboxylase
N
H
C
O
Lysyl residue
Site of CO$_2$ attachment
NH
O
=O
H-N
NH
H
H
S
H
Biotin
Biotin bound to an enzyme

Figure 28.16
A. Structure of biotin. B. Biotin covalently bound to a lysyl residue of a biotin-dependent enzyme.

X. PANTOTHENIC ACID

Pantothenic acid is a component of CoA, which functions in the transfer of acyl groups (Figure 28.17). CoA contains a thiol group that carries acyl compounds as activated thiol esters. Examples of such structures are succinyl CoA, fatty acyl CoA, and acetyl CoA. Pantothenic acid is also a component of the acyl carrier protein domain of fatty acid synthase (see p. 184). Eggs, liver, and yeast are the most important sources of pantothenic acid, although the vitamin is widely distributed. Pantothenic acid deficiency is not well characterized in humans, and no RDA has been established.

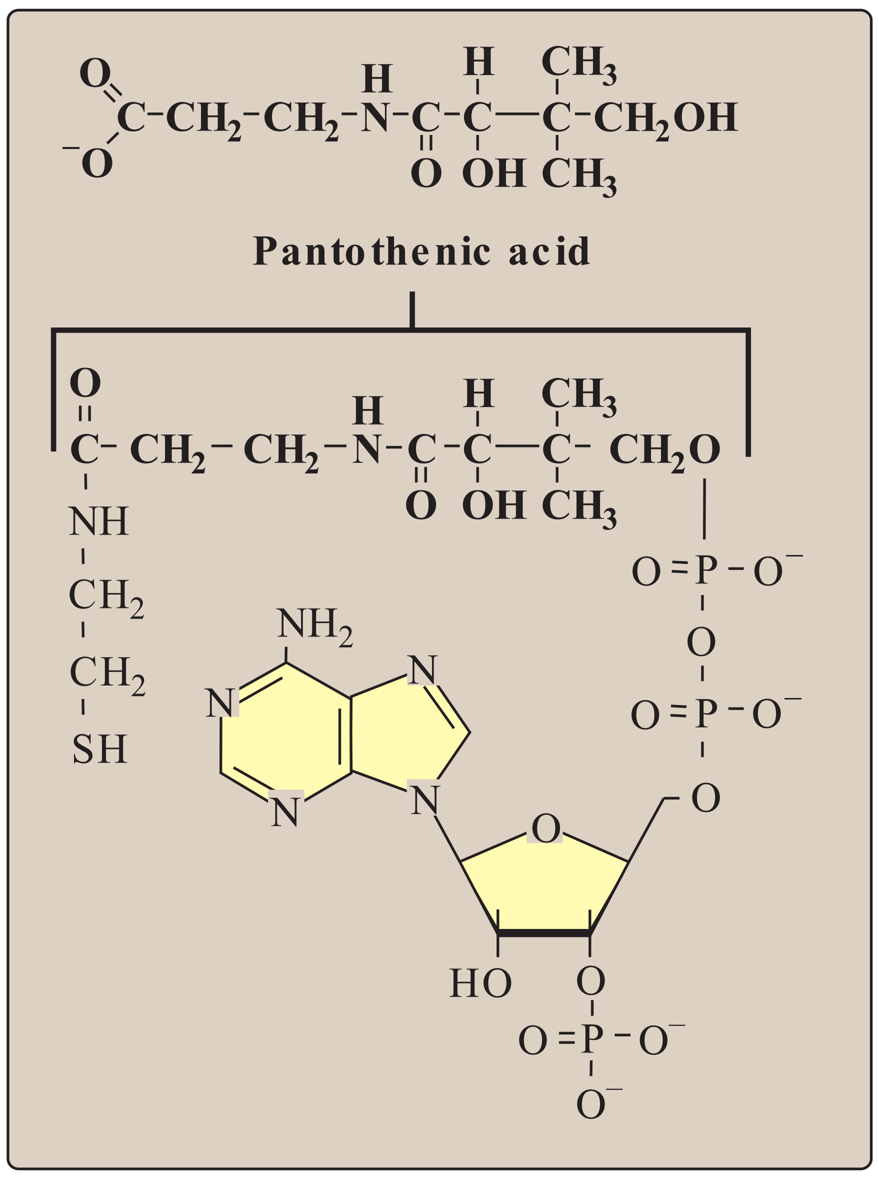

O
-O
C-CH$_2$-CH$_2$-N-C-C—C-CH$_2$OH
H
H CH$_3$
O OH CH$_3$
Pantothenic acid
O
C- CH$_2$- CH$_2$-N-C-C—C- CH$_2$O
H
H CH$_3$
O OH CH$_3$
NH
CH$_2$
CH$_2$
SH
O=P-O$^-$
O
O=P-O$^-$
O
NH$_2$
N
N
N
N
O
HO
O
O=P-O$^-$
O$^-$

Figure 28.17
Structure of coenzyme A.

XI. VITAMIN A

The retinoids, a family of molecules that are related to dietary retinol (vitamin A), are essential for vision, reproduction, growth, and maintenance of epithelial tissues. They also play a role in immune function. Retinoic acid, derived from oxidation of retinol, mediates most of the actions of the retinoids, except for vision, which depends on retinal, the aldehyde derivative of retinol.

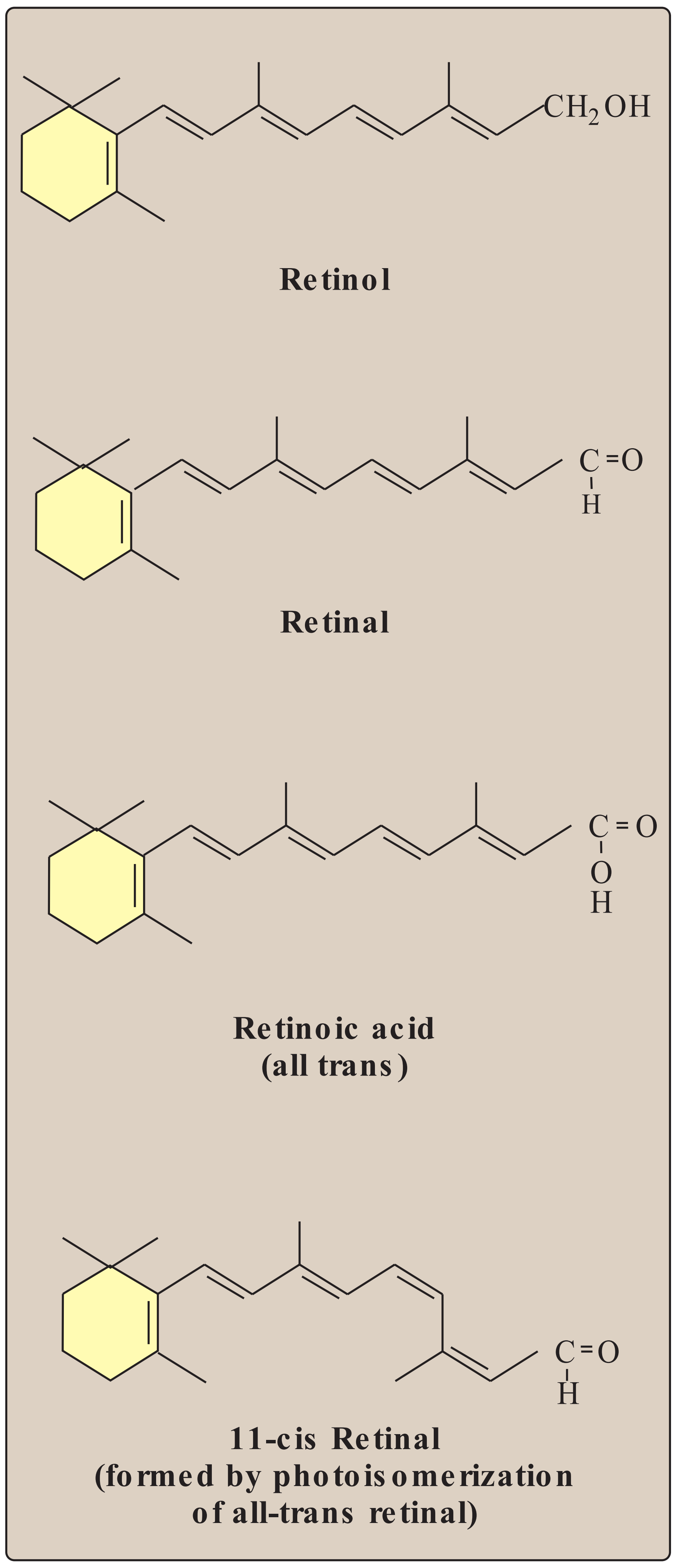


Figure 28.18
Structure of the retinoids.

A. Structure of vitamin A

Vitamin A is often used as a collective term for several related biologically active molecules (Figure 28.18). The term retinoids includes both natural and synthetic forms of vitamin A that may or may not show vitamin A activity.

1. **Retinol:** A primary alcohol containing a β-ionone ring with an unsaturated side chain, retinol is found in animal tissues as a retinyl ester with long-chain FAs.
2. **Retinal:** This is the aldehyde derived from the oxidation of retinol. Retinal and retinol can readily be interconverted.
3. **Retinoic acid:** This is the acid derived from the oxidation of retinal. Retinoic acid cannot be reduced in the body, and, therefore, cannot give rise to either retinal or retinol.
4. **β-Carotene:** Plant foods contain β-carotene, which can be oxidatively cleaved in the intestine to yield two molecules of retinal. In humans, the conversion is inefficient, and the vitamin A activity of β-carotene is only about 1/12 that of retinol.

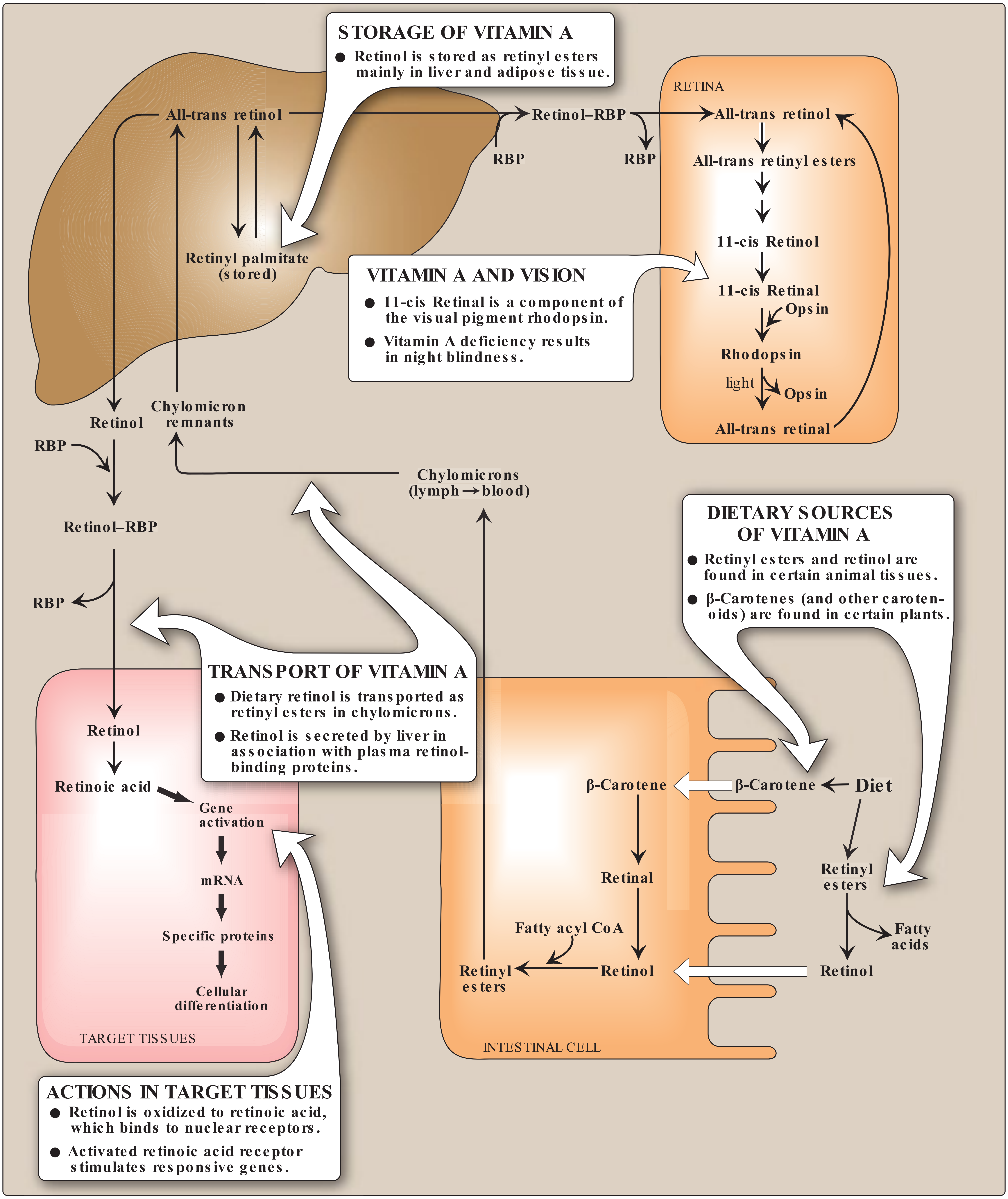
B. Absorption and transport of vitamin A

1. **Transport to the liver:** Retinyl esters present in the diet are hydrolyzed in the intestinal mucosa, releasing retinol and FFAs (Figure 28.19). Retinol derived from esters and from the cleavage and reduction of carotenes is re-esterified to long-chain FAs in the intestinal mucosa and secreted as a component of chylomicrons into the lymphatic system (see Figure 28.19). Retinyl esters contained in chylomicron remnants are taken up by, and stored in, the liver.

2. **Release from the liver:** When needed, retinol is released from the liver and transported to extrahepatic tissues by the plasma retinol-binding protein (RBP). The retinol–RBP complex binds to a transport protein on the surface of the cells of peripheral tissues, permitting retinol to enter. Many tissues contain a cellular retinol-binding protein that carries retinol to sites in the nucleus where the vitamin acts in a manner analogous to that of steroid hormones.

C. Mechanism of action of vitamin A

Retinol is oxidized to retinoic acid. Retinoic acid binds with high affinity to specific receptor proteins (retinoic acid receptors [RARs]) present in the nucleus of target tissues such as epithelial cells (Figure 28.20). The activated retinoic acid–RAR complex binds to response elements on DNA and recruits activators or repressors to regulate retinoid-specific RNA synthesis, resulting in control of the production of specific proteins that mediate several physiologic functions. For example, retinoids control the expression of the gene for keratin in most epithelial tissues of the body. The RAR proteins are part of the superfamily of transcriptional regulators that includes the nuclear receptors for steroid and thyroid hormones and 1,25-dihydroxycholecalciferol, all of which function in a similar way (see p. 240).


All-trans retinol
Retinyl palmitate (stored)
Retinol–RBP
RBP
RBP
RETINA
All-trans retinol
All-trans retinyl esters
11-cis Retinol
11-cis Retinal
Opsin
Rhodopsin
light
Opsin
All-trans retinal
Retinol
Chylomicron remnants
RBP
Retinol–RBP
RBP
Chylomicrons (lymph → blood)
Retinol
Retinoic acid
Gene activation
mRNA
Specific proteins
Cellular differentiation
TARGET TISSUES
β-Carotene
β-Carotene
Diet
Retinal
Retinyl esters
Fatty acids
Fatty acyl CoA
Retinyl esters
Retinol
Retinol
INTESTINAL CELL

STORAGE OF VITAMIN A

- Retinol is stored as retinyl esters mainly in liver and adipose tissue.

VITAMIN A AND VISION

- 11-cis Retinal is a component of the visual pigment rhodopsin.
- Vitamin A deficiency results in night blindness.

DIETARY SOURCES OF VITAMIN A

- Retinyl esters and retinol are found in certain animal tissues.
- β-Carotenes (and other carotenoids) are found in certain plants.

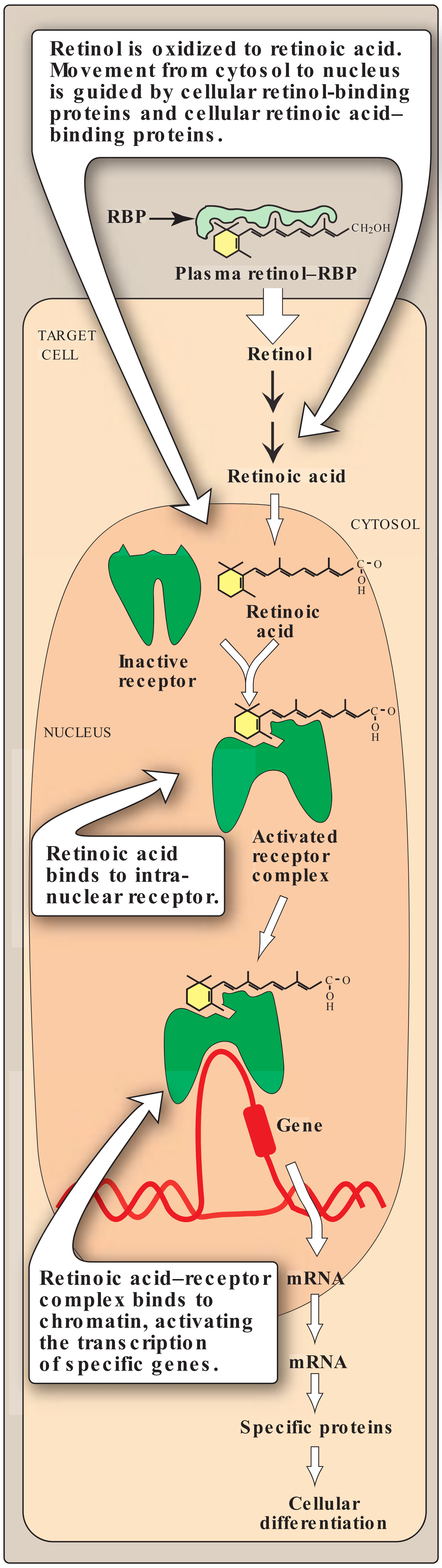
TRANSPORT OF VITAMIN A

- Dietary retinol is transported as retinyl esters in chylomicrons.
- Retinol is secreted by liver in association with plasma retinol-binding proteins.

ACTIONS IN TARGET TISSUES

- Retinol is oxidized to retinoic acid, which binds to nuclear receptors.
- Activated retinoic acid receptor stimulates responsive genes.

Figure 28.19
Absorption, transport, and storage of vitamin A and its derivatives. RBP = retinol-binding protein; CoA = coenzyme A; mRNA = messenger RNA.



Retinol is oxidized to retinoic acid. Movement from cytosol to nucleus is guided by cellular retinol-binding proteins and cellular retinoic acid–binding proteins.

Retinoic acid binds to intra-nuclear receptor.

Retinoic acid–receptor complex binds to chromatin, activating the transcription of specific genes.

Figure 28.20

Action of the retinoids. [Note: Retinoic acid–receptor complex forms a dimer, but is shown as monomer for simplicity.] RBP = retinol-binding protein; mRNA = messenger RNA.

D. Functions of vitamin A

1. **Visual cycle:** Vitamin A is a component of the visual pigments of rod and cone cells. Rhodopsin, the visual pigment of the rod cells in the retina, consists of 11-cis retinal specifically bound to the protein opsin. When rhodopsin is exposed to light, a series of photochemical isomerizations occurs, which results in the bleaching of the visual pigment and release of all-trans retinal and opsin. This process triggers a nerve impulse that is transmitted by the optic nerve to the brain. Regeneration of rhodopsin requires isomerization of all-trans retinal back to 11-cis retinal. All-trans retinal, after being released from rhodopsin, is reduced to all-trans retinol, esterfied, and isomerized to 11-cis retinol that is oxidized to 11-cis retinal. The latter combines with opsin to form rhodopsin, thus completing the cycle. Similar reactions are responsible for color vision in the cone cells.

2. **Maintenance of epithelial cells:** Vitamin A is essential for normal differentiation of epithelial tissues and mucus secretion, and thus, supports the body's barrier-based defense against pathogens.

3. **Reproduction:** Retinol and retinal are essential for normal reproduction, supporting spermatogenesis in the male and preventing fetal resorption in the female. Retinoic acid is inactive in maintaining reproduction and in the visual cycle but promotes growth and differentiation of epithelial cells. Therefore, animals given vitamin A only as retinoic acid from birth are blind and sterile.

E. Distribution of vitamin A

Liver, kidney, cream, butter, and egg yolk are good sources of preformed vitamin A. Yellow, orange, and dark green vegetables and fruits are good dietary sources of the carotenes, which serve as precursors of vitamin A.

F. Requirement for vitamin A

The RDA for adults is 900 retinol activity equivalents (RAEs) for males and 700 RAE for females. In comparison, 1 RAE = 1 mg of retinol, 12 mg of β-carotene, or 24 mg of other carotenoids.

G. Clinical indications

Although chemically related, retinoic acid and retinol have distinctly different therapeutic applications. Retinol and its carotenoid precursor are used as dietary supplements, whereas various forms of retinoic acid are useful in dermatology.

1. **Dietary deficiency:** Vitamin A, administered as retinol or retinyl esters, is used to treat patients who are deficient in the vitamin (Figure 28.21). Night blindness is one of the earliest signs of vitamin A deficiency. The visual threshold is increased, making it difficult to see in dim light. Prolonged deficiency leads to an irreversible loss in the number of visual cells. Severe vitamin A deficiency leads to xerophthalmia, a pathologic dryness of the conjunctiva and cornea, caused, in part, by increased keratin synthesis. If untreated, xerophthalmia results in corneal ulceration and, ultimately, in blindness because of the formation of opaque scar tissue. The condition is most commonly seen in children in developing tropical countries. Over 500,000 children worldwide are blinded each year by xerophthalmia caused by insufficient vitamin A in the diet.

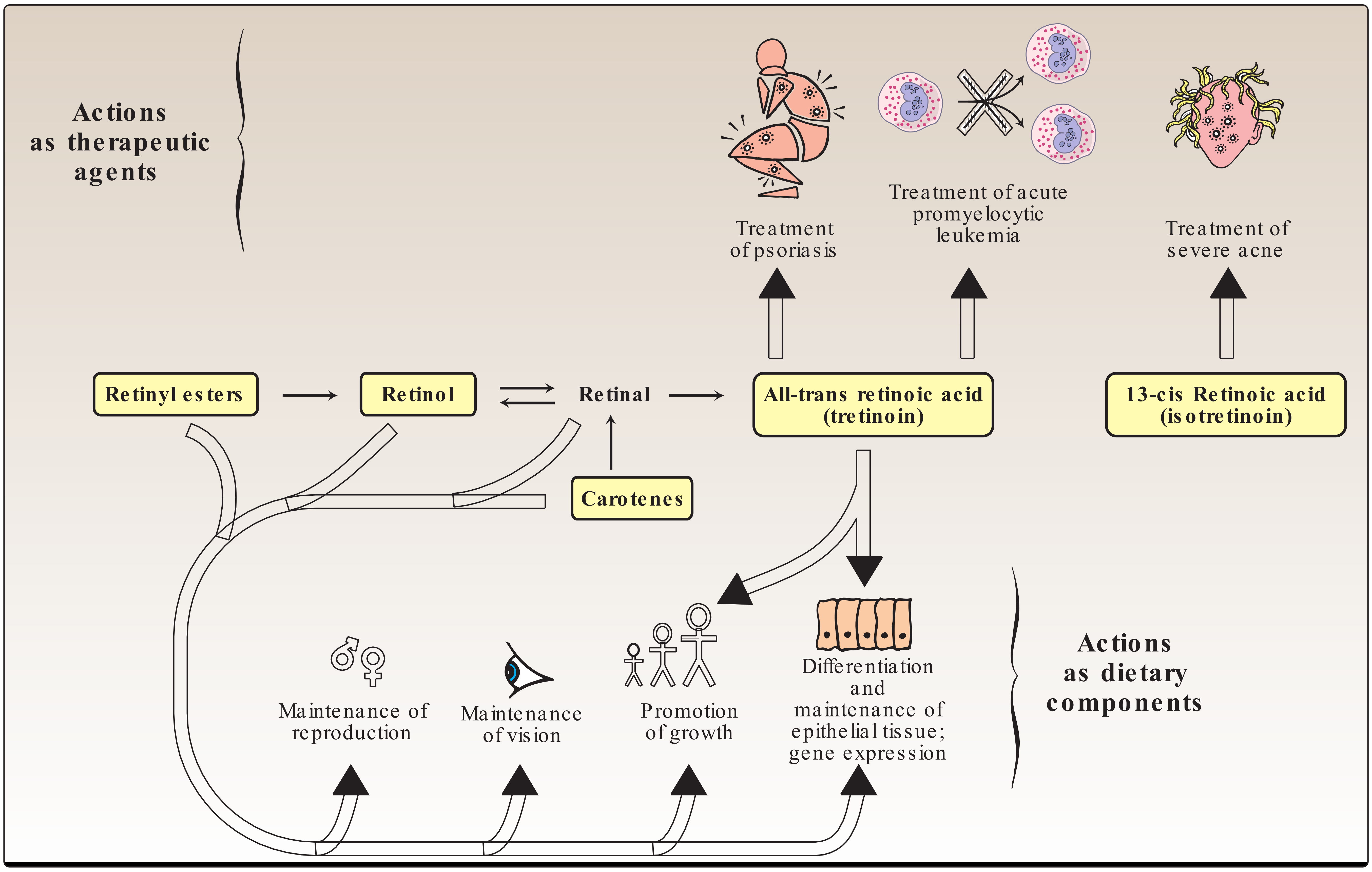

Actions as therapeutic agents
Treatment of psoriasis
Treatment of acute promyelocytic leukemia
Treatment of severe acne
Retinyl esters
Retinol
Retinal
All-trans retinoic acid (tretinoin)
13-cis Retinoic acid (isotretinoin)
Carotenes
Maintenance of reproduction
Maintenance of vision
Promotion of growth
Differentiation and maintenance of epithelial tissue; gene expression
Actions as dietary components

Figure 28.21
Summary of actions of retinoids. Compounds in boxes are available as dietary components or as pharmacologic agents.

2. **Acne and psoriasis:** Dermatologic problems such as acne and psoriasis are effectively treated with retinoic acid or its derivatives (see Figure 28.21). Mild cases of acne, Darier disease (keratosis follicularis), and skin aging are treated with topical application of tretinoin (all-trans retinoic acid), as well as benzoyl peroxide and antibiotics. [Note: Tretinoin is too toxic for systemic administration and is confined to topical application.] In patients with severe cystic acne unresponsive to conventional therapies, the drug of choice is isotretinoin (13-cis retinoic acid) administered orally. Retinoic acid is also used in the treatment of acute promyelocytic leukemia.

H. Toxicity of retinoids

1. **Vitamin A:** Excessive intake of vitamin A produces a toxic syndrome called hypervitaminosis A. Amounts exceeding 7.5 mg/day of retinol should be avoided. Early signs of chronic hypervitaminosis A are reflected in the skin, which becomes dry and pruritic (due to decreased keratin synthesis); the liver, which becomes enlarged and can become cirrhotic; and in the CNS, where a rise in intracranial pressure may mimic the symptoms of a brain tumor. Pregnant women particularly should not ingest excessive quantities of vitamin A because of its potential for teratogenesis (causing congenital malformations in the developing fetus). UL is 3,000 mg/day. [Note: Vitamin A promotes bone growth. In

excess, however, it is associated with decreased bone mineral density and increased risk of fractures.]

2. **Isotretinoin:** The drug, an isomer of retinoic acid, is teratogenic and absolutely contraindicated in women with childbearing potential unless they have severe, disfiguring cystic acne that is unresponsive to standard therapies. Pregnancy must be excluded before initiation of treatment, and adequate birth control must be used. Prolonged treatment with isotretinoin leads to hyperlipidemia with an increase in TAGs and cholesterol, providing some concern for an increased risk of cardiovascular disease.

XII. VITAMIN D

The D vitamins are a group of sterols that have a hormone-like function. The active molecule, 1,25-dihydroxycholecalciferol ([1,25-diOH-D_3] calcitriol), binds to intracellular receptor proteins. The 1,25-diOH-D_3–receptor complex interacts with DNA in the nucleus of target cells in a manner similar to that of vitamin A (see Figure 28.20) and either selectively stimulates or represses gene transcription. The most prominent actions of 1,25-diOH-D_3 are to regulate the plasma levels of calcium and phosphorus.

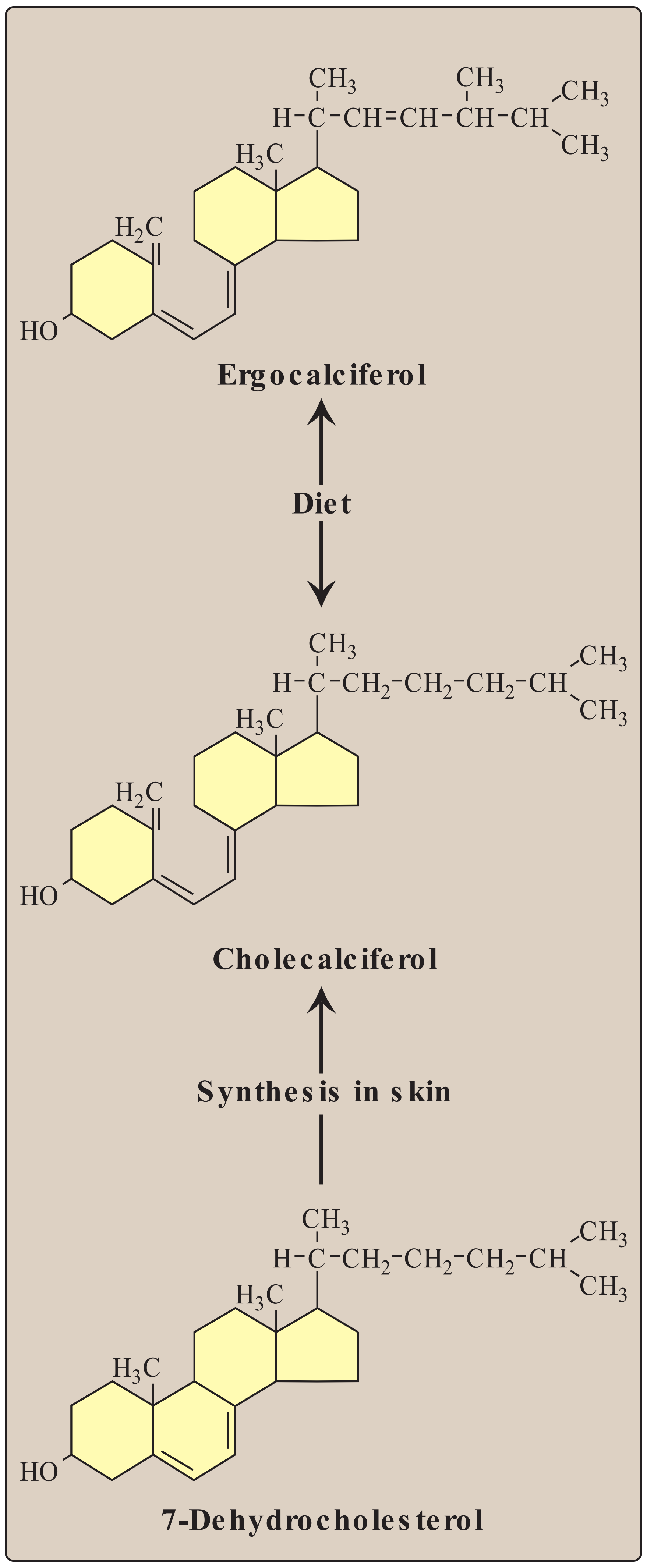


Figure 28.22
Sources of vitamin D.

A. Distribution of vitamin D

1. **Endogenous vitamin precursor:** 7-Dehydrocholesterol, an intermediate in cholesterol synthesis, is converted to cholecalciferol in the dermis and epidermis of humans exposed to sunlight and transported to liver bound to vitamin D–binding protein.

2. **Diet:** Ergocalciferol (vitamin D_2), found in plants, and cholecalciferol (vitamin D_3), found in animal tissues, are sources of preformed vitamin D activity (Figure 28.22). Ergocalciferol and cholecalciferol differ chemically only in the presence of an additional double bond and methyl group in the plant sterol. Dietary vitamin D is packaged into chylomicrons. [Note: Preformed vitamin D is a dietary requirement only in individuals with limited exposure to sunlight.]

B. Metabolism of vitamin D

1. **Formation of 1,25-dihydroxycholecalciferol:** Vitamins D_2 and D_3 are not biologically active but are converted in vivo to the active form of the D vitamin by two sequential hydroxylation reactions (Figure 28.23). The first hydroxylation occurs at the 25 position and is catalyzed by a specific 25-hydroxylase in the liver. The product of the reaction, 25-hydroxycholecalciferol ([25-OH-D_3], calcidiol), is the predominant form of vitamin D in the plasma and the major storage form of the vitamin. 25-OH-D_3 is further hydroxylated at the 1 position by 25-hydroxycholecalciferol 1-hydroxylase found primarily in the kidney, resulting in the formation of 1,25-diOH-D_3 (calcitriol). [Note: This 1-hydroxylase, as well as the liver 25-hydroxylase, are cytochrome P450 (CYP) proteins (see p. 149).]

2. **Regulation of 25-hydroxycholecalciferol 1-hydroxylase:** 1,25-diOH-D_3 is the most potent vitamin D metabolite. Its formation is tightly regulated by the level of plasma phosphate and calcium

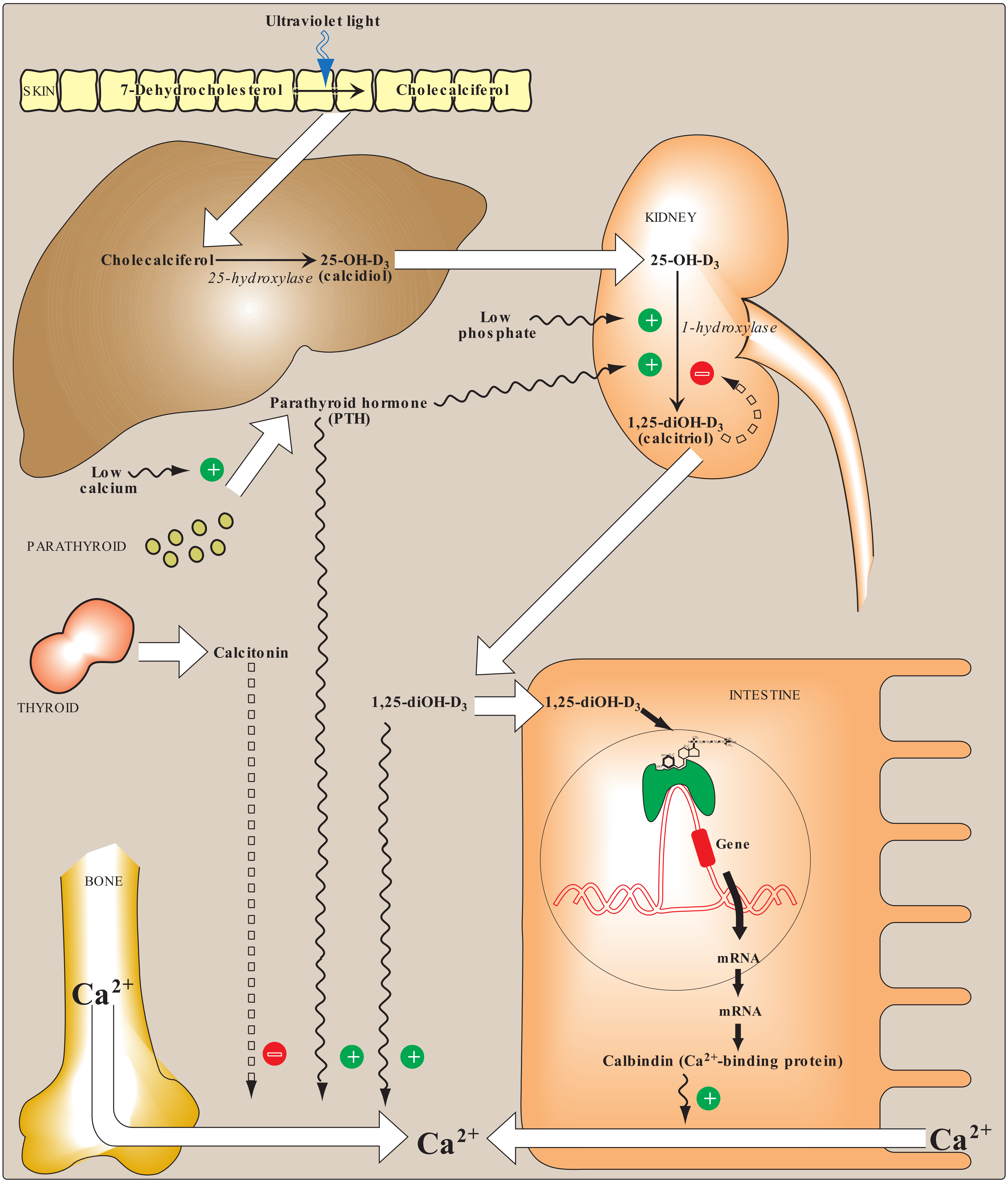

Ultraviolet light
SKIN
7-Dehydrocholesterol
Cholecalciferol
Cholecalciferol
25-hydroxylase
25-OH-D3 (calcidiol)
KIDNEY
25-OH-D3
Low phosphate
1-hydroxylase
Parathyroid hormone (PTH)
1,25-diOH-D3 (calcitriol)
Low calcium
PARATHYROID
THYROID
Calcitonin
1,25-diOH-D3
1,25-diOH-D3
INTESTINE
Gene
mRNA
mRNA
Calbindin (Ca2+-binding protein)
BONE
Ca2+
Ca2+
Ca2+

Figure 28.23
Metabolism and actions of vitamin D. [Note: Calcitonin, a thyroid hormone, decreases blood calcium by inhibiting mobilization from bone, absorption from the intestine, and reabsorption by the kidney. It opposes the actions of PTH.] mRNA = messenger RNA; 25-OH-D3 = 25-hydroxycholecalciferol; 1,25-diOH-D3 = 1, 25-dihydroxycholecalciferol.

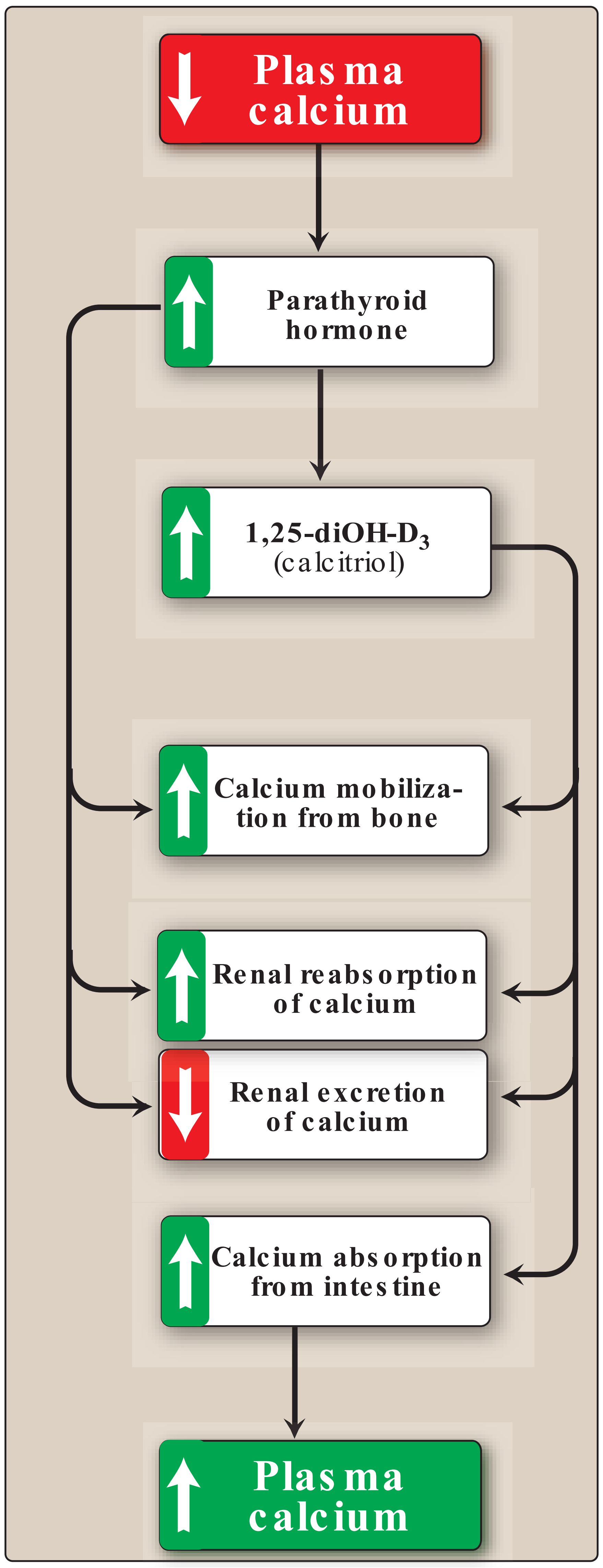

Plasma calcium
Parathyroid hormone
1,25-diOH-D3 (calcitriol)
Calcium mobilization from bone
Renal reabsorption of calcium
Renal excretion of calcium
Calcium absorption from intestine
Plasma calcium

Figure 28.24
Response to low plasma calcium. 1,25-diOH-D_3 = 1,25-dihydroxycholecalciferol.

ions (Figure 28.24). 25-Hydroxycholecalciferol 1-hydroxylase activity is increased directly by low plasma phosphate or indirectly by low plasma calcium, which triggers the secretion of parathyroid hormone (PTH) from the chief cells of the parathyroid gland. PTH upregulates the 1-hydroxylase. Thus, hypocalcemia caused by insufficient dietary calcium results in elevated levels of plasma 1,25-diOH-D_3. [Note: 1,25-diOH-D_3 inhibits synthesis of PTH, forming a negative feedback loop. It also inhibits activity of the 1-hydroxylase.]

C. Function of vitamin D

The overall function of 1,25-diOH-D_3 is to maintain adequate plasma levels of calcium. It performs this function by: 1) increasing uptake of calcium by the intestine, 2) minimizing loss of calcium by the kidney by increasing reabsorption, and 3) stimulating resorption (demineralization) of bone when blood calcium is low (see Figure 28.23).

1. **Effect of vitamin D on the intestine:** 1,25-diOH-D_3 stimulates intestinal absorption of calcium. 1,25-diOH-D_3 enters the intestinal cell and binds to a cytosolic receptor. The 1,25-diOH-D_3–receptor complex then moves to the nucleus where it selectively interacts with response elements on the DNA. As a result, calcium uptake is enhanced by an increased synthesis of a specific calcium-binding protein, calbindin. Thus, the mechanism of action of 1,25-diOH-D_3 is typical of steroid hormones (see p. 240).

2. **Effect of vitamin D on bone:** 1,25-diOH-D_3 stimulates the mobilization of calcium from bone by a process that requires protein synthesis and the presence of PTH. The result is an increase in plasma calcium and phosphate. Therefore, bone is an important reservoir of calcium that can be mobilized to maintain plasma levels. [Note: PTH and calcitriol also work together to prevent renal loss of calcium.]

D. Distribution and requirement of vitamin D

Vitamin D occurs naturally in fatty fish, liver, and egg yolk. Milk, unless it is artificially fortified, is not a good source of the vitamin. The RDA for individuals ages 1 to 70 years is 15 mg/day and 20 mg/day if over age 70 years. Experts disagree, however, on the optimal level of vitamin D needed to maintain health. [Note: 1 mg = 40 international units (IUs).] Because breast milk is a poor source of vitamin D, supplementation is recommended for breastfed babies.

E. Clinical indications

1. **Nutritional rickets:** Vitamin D deficiency causes a net demineralization of bone, resulting in rickets in children and osteomalacia in adults (Figure 28.25). Rickets is characterized by the continued formation of the collagen matrix of bone, but incomplete mineralization results in soft, pliable bones. In osteomalacia, demineralization of pre-existing bones increases their susceptibility to fracture. Insufficient exposure to daylight and/or deficiencies in vitamin D consumption occur predominantly in infants and the elderly. Vitamin D deficiency is more common in the northern latitudes, because less vitamin D synthesis occurs in the skin as a result of reduced exposure to ultraviolet light.

2. **Renal osteodystrophy:** Chronic kidney disease causes decreased ability to form active vitamin D as well as increased retention of phosphate, resulting in hyperphosphatemia and hypocalcemia. The low blood calcium causes a rise in PTH and associated bone demineralization with release of calcium and phosphate. Supplementation with calcitriol is an effective therapy. However, supplementation must be accompanied by phosphate reduction therapy to prevent further bone loss and precipitation of calcium phosphate crystals.

3. **Hypoparathyroidism:** Lack of PTH causes hypocalcemia and hyperphosphatemia. These patients may be treated with calcitriol and calcium supplementation.

F. Toxicity of vitamin D

Like all fat-soluble vitamins, vitamin D can be stored in the body and is only slowly metabolized. High doses (100,000 IUs for weeks or months) can cause loss of appetite, nausea, thirst, and stupor. Enhanced calcium absorption and bone resorption results in hypercalcemia, which can lead to deposition of calcium in many organs, particularly the arteries and kidneys. The UL is 100 mg/day (4,000 IU/day) for individuals ages 9 years or older, with a lower level for those under age 9 years. [Note: Toxicity is only seen with use of supplements. Excess vitamin D produced in the skin is converted to inactive forms.]

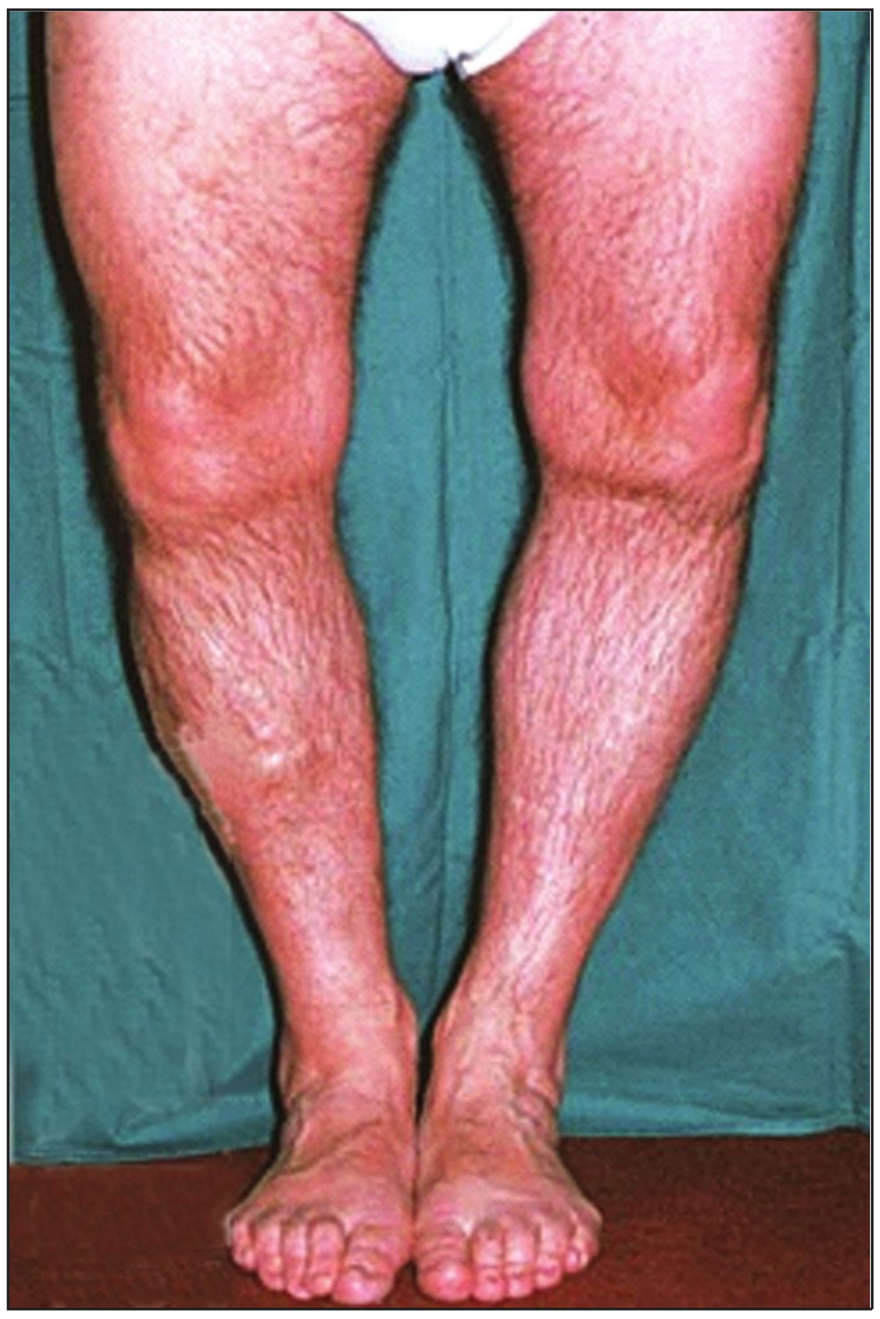


Figure 28.25
Bowed legs of middle-aged man with osteomalacia, a nutritional vitamin D deficiency that results in demineralization of the skeleton.

XIII. VITAMIN K

The principal role of vitamin K is in the posttranslational modification of a number of proteins (most of which are involved with blood clotting), in which it serves as a coenzyme in the carboxylation of certain glutamic acid residues present in these proteins. Vitamin K exists in several forms, for example, in plants as phylloquinone (or vitamin K_1), and in intestinal bacterial flora as menaquinone (or vitamin K_2). A synthetic form of vitamin K, menadione, is able to be converted to K_2.

A. Function of vitamin K

1. **Formation of γ-carboxyglutamate:** Vitamin K is required in the hepatic synthesis of the blood clotting proteins, prothrombin (factor II) and factors VII, IX, and X. (See online Chapter 34.) Formation of the functional clotting factors requires the vitamin K–dependent carboxylation of several glutamic acid residues to γ-carboxyglutamate (Gla) residues (Figure 28.26). The carboxylation reaction requires γ-glutamyl carboxylase, O_2, CO_2, and the hydroquinone form of vitamin K (which gets oxidized to the epoxide form). The formation of Gla residues is sensitive to inhibition by warfarin, a synthetic analog of vitamin K that inhibits vitamin K epoxide reductase (VKOR), the enzyme required to regenerate the functional hydroquinone form of vitamin K.

2. **Interaction of prothrombin with membranes:** The Gla residues are good chelators of positively charged calcium ions, because of their two adjacent, negatively charged carboxylate groups. With prothrombin, for example, the prothrombin–calcium complex is able to bind to negatively charged membrane phospholipids on the surface of damaged endothelium and platelets. Attachment to

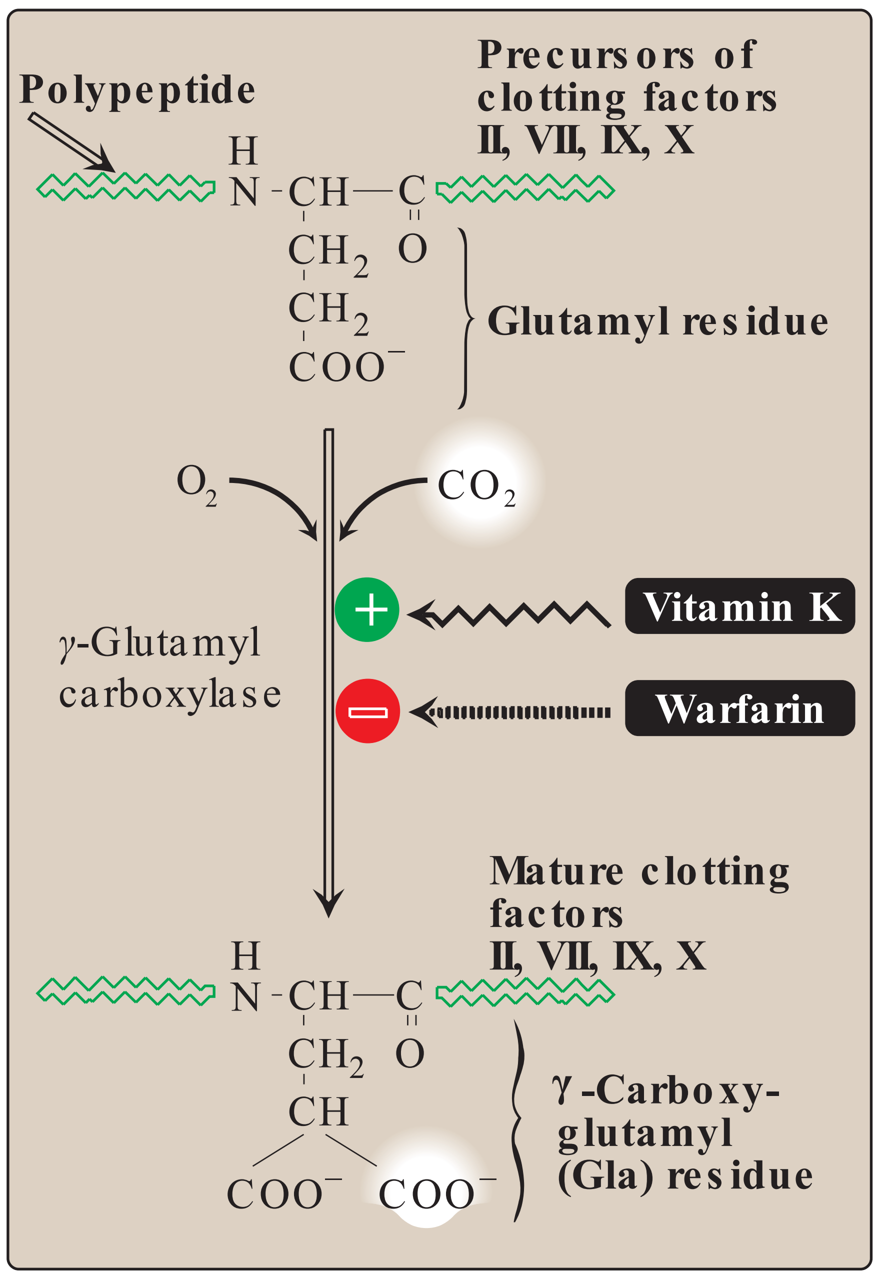


Figure 28.26
Carboxylation of glutamate to form γ-carboxyglutamate.

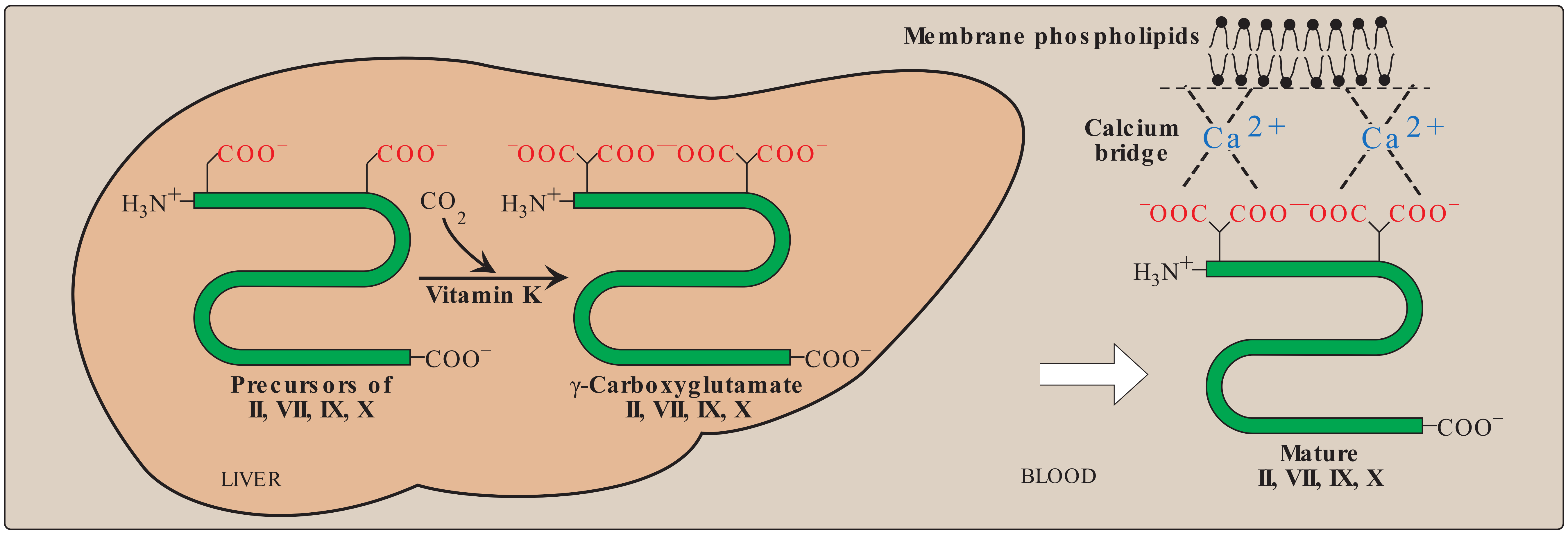


Figure 28.27
Role of vitamin K in blood coagulation.

membrane increases the rate at which the proteolytic conversion of prothrombin to thrombin can occur (Figure 28.27).

3. **γ-Carboxyglutamate residues in other proteins:** Gla residues are also present in proteins other than those involved in forming a blood clot. For example, osteocalcin of bone and proteins C and S (involved in limiting the formation of blood clots) also undergo γ-carboxylation.

B. Distribution and requirement of vitamin K

Vitamin K is found in cabbage, kale, spinach, egg yolk, and liver. There is also extensive synthesis of the vitamin by the bacteria in the gut. The adequate intake for vitamin K is 120 mg/day for adult males and 90 mg for adult females.

C. Clinical indications

1. **Deficiency of vitamin K:** A true vitamin K deficiency is unusual because adequate amounts are generally produced by intestinal bacteria or obtained from the diet. If the bacterial population in the gut is decreased (for example, by antibiotics), the amount of endogenously formed vitamin is depressed, and this can lead to hypoprothrombinemia in the marginally malnourished individual (for example, a debilitated geriatric patient). This condition may require supplementation with vitamin K to correct the bleeding tendency. In addition, certain second-generation cephalosporin antibiotics (for example, cefamandole) cause hypoprothrombinemia, apparently by a warfarin-like mechanism that inhibits VKOR. Consequently, their use in treatment is usually supplemented with vitamin K.

2. **Deficiency of vitamin K in the newborn:** Newborns have sterile intestines and, so, initially lack the bacteria that synthesize vitamin K. Because human milk provides only about one fifth of the daily requirement for vitamin K, it is recommended that all newborns receive a single intramuscular dose of vitamin K as prophylaxis against hemorrhagic disease.

D. Toxicity of vitamin K

Prolonged administration of large doses of synthetic vitamin K (menadione) can produce hemolytic anemia and jaundice in the infant, due to toxic effects on the membrane of RBCs. Therefore, it is no longer used to treat vitamin K deficiency. No UL has been set for vitamin K.

XIV. VITAMIN E

The E vitamins consist of eight naturally occurring tocopherols, of which α-tocopherol is the most active (Figure 28.28). The primary function of vitamin E is as an antioxidant in prevention of the nonenzymic oxidation of cell components (for example, peroxidation of polyunsaturated FAs by molecular oxygen and free radicals).

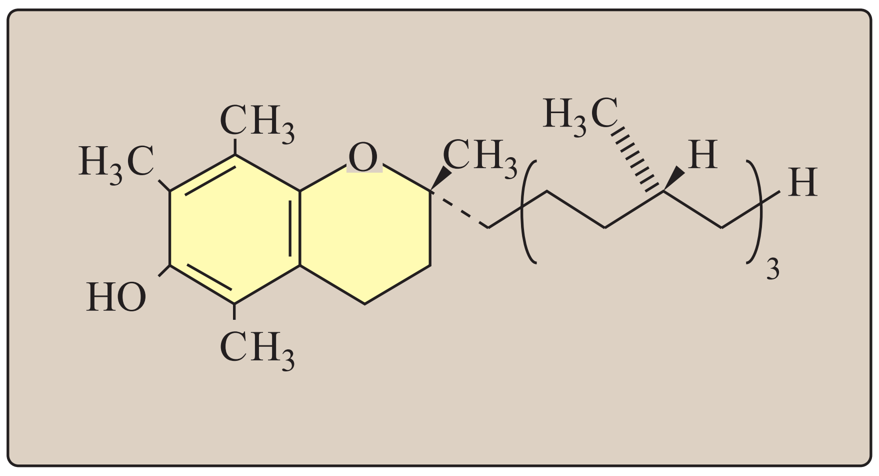


Figure 28.28
Structure of vitamin E.

A. Distribution and requirements of vitamin E

Vegetable oils are rich sources of vitamin E, whereas liver and eggs contain moderate amounts. The RDA for α-tocopherol is 15 mg/day for adults. The vitamin E requirement increases as the intake of polyunsaturated FA increases to limit FA peroxidation.

B. Deficiency of vitamin E

Newborns have low reserves of vitamin E, but breast milk (and formulas) contain the vitamin. Very-low-birth-weight infants may be given supplements to prevent the hemolysis and retinopathy associated with deficiency of vitamin E. When observed in adults, deficiency is usually associated with defective lipid absorption or transport. [Note: Abetalipoproteinemia, caused by a defect in the formation of chylomicrons (and VLDL), results in vitamin E deficiency (see p. 231).]

C. Clinical indications

Vitamin E is not recommended for the prevention of chronic disease, such as coronary heart disease or cancer. Clinical trials using vitamin E supplementation have been uniformly disappointing. For example, subjects in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study trial who received high doses of vitamin E not only lacked cardiovascular benefit but also had an increased incidence of stroke.

D. Toxicity of vitamin E

Vitamin E is the least toxic of the fat-soluble vitamins, and no toxicity has been observed at doses of 300 mg/day (UL = 1,000 mg/day).

> Populations consuming diets high in fruits and vegetables show decreased incidence of some chronic diseases. However, clinical trials have failed to show a definitive benefit from supplements of vitamins A, C, or E; multivitamins with folic acid; or antioxidant combinations for the prevention of cancer or cardiovascular disease.

The vitamins are summarized in Figure 28.29.

VITAMIN	OTHER NAMES	ACTIVE FORM	FUNCTION
Folic acid	—	Tetrahydro-folic acid	Transfer one-carbon units; synthesis of methionine, purines, and thymidine monophosphate
Vitamin B_{12}	Cobalamin	Methylcobalamin Deoxyadenosyl cobalamin	Coenzyme for reactions: Homocysteine → methionine Methylmalonyl CoA → succinyl CoA
Vitamin C	Ascorbic acid	Ascorbic acid	Antioxidant Coenzyme for hydroxylation reactions, for example: In procollagen: Proline → hydroxyproline Lysine → hydroxylysine
Vitamin B_6	Pridoxine Pyridoxamine Pyridoxal	Pyridoxal phosphate	Coenzyme for enzymes, particularly in amino acid metabolism
Vitamin B_1	Thiamine	Thiamine pyrophosphate	Coenzyme of enzymes catalyzing: Pyruvate → acetyl CoA α-Ketoglutarate → Succinyl CoA Ribose 5-P + xylulose 5-P → Sedoheptulose 7-P + Glyceraldehyde 3-P Branched-chain α-keto acid oxidation
Niacin	Nicotinic acid Nicotinamide	NAD^+, $NADP^+$	Electron transfer
Vitamin B_2	Riboflavin	FMN, FAD	Electron transfer
Biotin	—	Enzyme-bound biotin	Carboxylation reactions
Pantothenic acid	—	Coenzyme A	Acyl carrier WATER SOLUBLE
Vitamin A	Retinol Retinal Retinoic acid β-Carotene	Retinol Retinal Retinoic acid	FAT SOLUBLE Maintenance of reproduction Vision Promotion of growth Differentiation and maintenance of epithelial tissues Gene expression
Vitamin D	Cholecalciferol Ergocalciferol	1,25-Dihydroxy-cholecalciferol	Calcium uptake Gene expression
Vitamin K	Menadione Menaquinone Phylloquinone	Menadione Menaquinone Phylloquinon	γ-Carboxylation of glutamate residues in clotting and other proteins
Vitamin E	α-Tocopherol	Any of several tocopherol derivatives	Antioxidant

Figure 28.29 (continued on next page)
Summary of vitamins. P = phosphate; NAD(P) = nicotinamide adenine dinucleotide (phosphate); FMN = flavin mononucleotide; FAD = flavin adenine dinucleotide; CoA = coenzyme A.

DEFICIENCY	SIGNS AND SYMPTOMS	TOXICITY	NOTES
Megaloblastic anemia Neural tube defects	Anemia Birth defects	None	Administration of high levels of folate can mask vitamin B_{12} deficiency
Pernicious anemia Dementia Spinal degeneration	Megaloblastic anemia Neuropsychiatric symptoms	None	Pernicious anemia is treated with intramuscular or high-dose oral vitamin B_{12}
Scurvy	Sore, spongy gums Loose teeth Poor wound healing	None	Benefits of supplementation not established in controlled trials
Rare	Glossitis Neuropathy	Yes	Deficiency can be induced by isoniazid Sensory neuropathy occurs at high doses
Beriberi Wernicke-Korsakoff syndrome (most common in alcoholics)	Tachycardia, vomiting, convulsions Apathy, loss of memory, dysregulated eye movements	None	—
Pellagra	Dermatitis Diarrhea Dementia	None	High doses of niacin used to treat hyperlipidemia
Rare	Dermatitis Angular stomatitis	None	—
Rare	—	None	Consumption of large amounts of raw egg whites (which contains a protein, avidin, that binds biotin) can induce a biotin deficiency
Rare	—	None	—
			WATER SOLUBLE
			FAT SOLUBLE
infertility Night blindness Retardation of growth Xerophthalmia	Increased visual threshold Dryness of cornea	Yes	β-Carotene not acutely toxic, but supplementation is not recommended Excess vitamin A can increase incidence of fractures
Rickets (in children) Osteomalacia (in adults)	Soft, pliable bones	Yes	Vitamin D is not a true vitamin because it can be synthesized in skin; application of sunscreen lotions or presence of dark skin color decreases this synthesis.
Newborn Rare in adults	Bleeding	Rare	Vitamin K produced by intestinal bacteria. Vitamin K deficiency common in newborns Intramuscular treatment with vitamin K is recommended at birth
Rare	Red blood cell fragility leads to hemolytic anemia	None	Benefits of supplementation not established in controlled trials

Figure 28.29 (continued from previous page)
Summary of vitamins.

UNIT VI:
Storage and Expression of Genetic Information

DNA Structure, Replication, and Repair

29

I. OVERVIEW

Nucleic acids are required for the storage and expression of genetic information. There are two chemically distinct types of nucleic acids: deoxyribonucleic acid (DNA) and ribonucleic acid ([RNA], see Chapter 30). DNA, the repository of genetic information, is present not only in chromosomes in the nucleus of eukaryotic organisms, but also in mitochondria and the chloroplasts of plants. Prokaryotic cells, which lack nuclei, have a single chromosome but may also contain nonchromosomal DNA in the form of plasmids. The genetic information found in DNA is copied and transmitted to daughter cells through DNA replication. The DNA contained in a fertilized egg encodes the information that directs the development of an organism. This development may involve the production of billions of cells. Each cell is specialized, expressing only those functions that are required for it to perform its role in maintaining the organism. Therefore, DNA must be able to not only replicate precisely each time a cell divides, but also to have the information that it contains be selectively expressed. Transcription (RNA synthesis) is the first stage in the expression of genetic information (see Chapter 30). Next, the code contained in the nucleotide sequence of messenger RNA molecules is translated (protein synthesis; see Chapter 31), thus completing gene expression. The regulation of gene expression is discussed in Chapter 32.

> The flow of information from DNA to RNA to protein is termed the "central dogma" of molecular biology (Figure 29.1) and is descriptive of all organisms, with the exception of some viruses that have RNA as the repository of their genetic information.

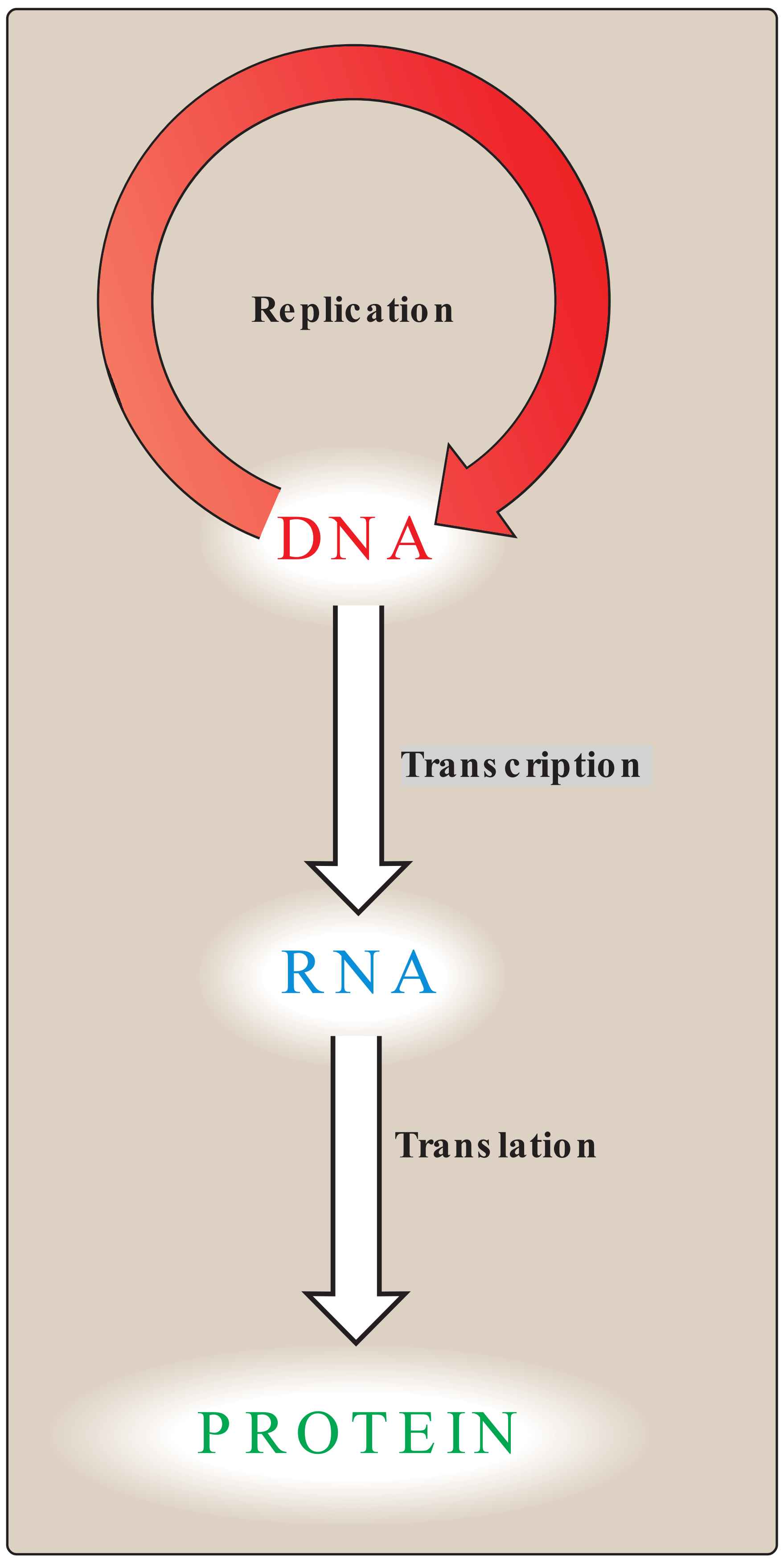


Figure 29.1
The "central dogma" of molecular biology.

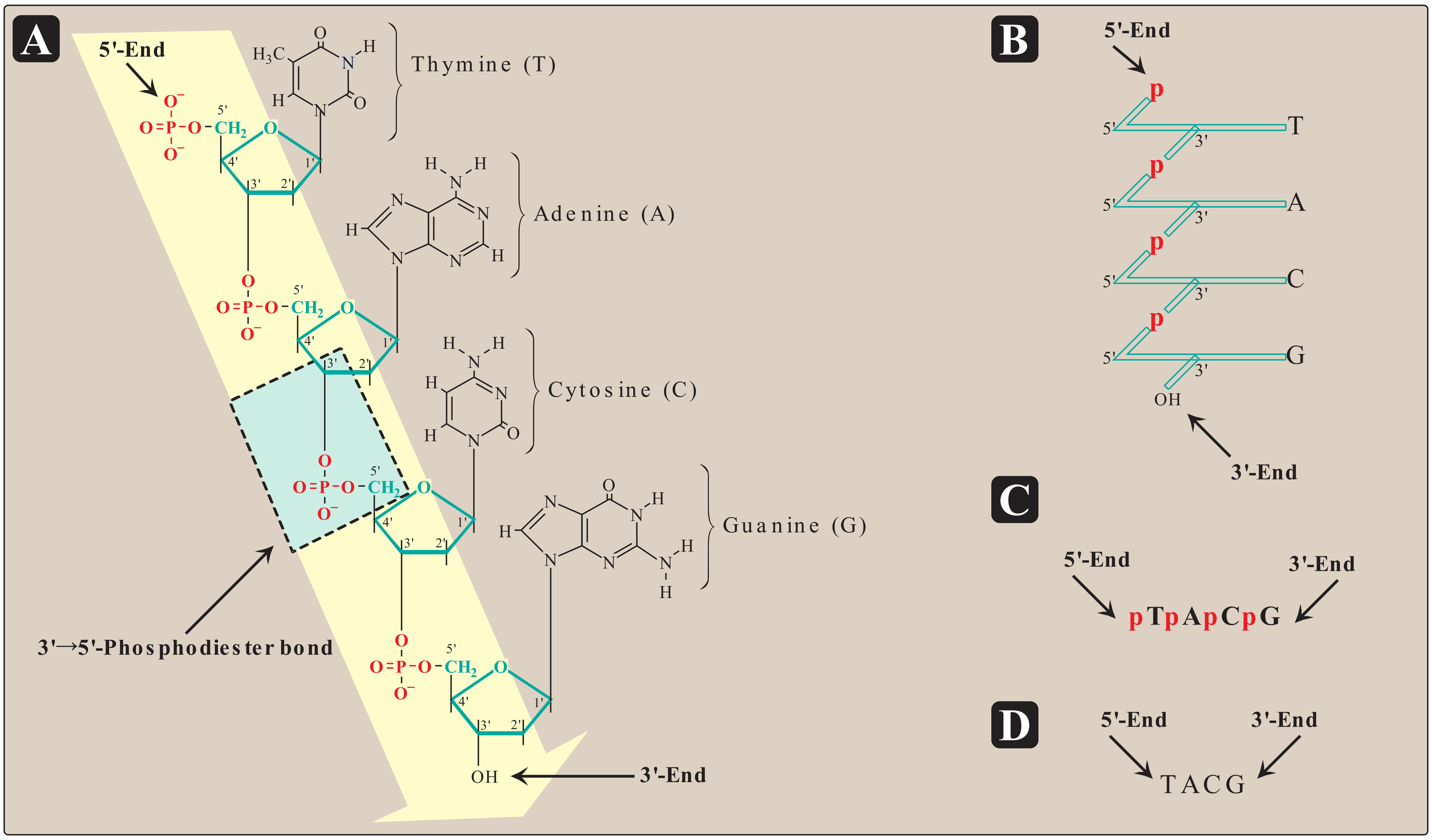


Figure 29.2
A. DNA chain with the nucleotide sequence shown written in the 5'→3' direction. A 3'→5'-phosphodiester bond is shown highlighted in the blue box, and the deoxyribose-phosphate backbone is shaded in yellow. B. The DNA chain written in a more stylized form, emphasizing the deoxyribose–phosphate backbone. C. A simpler representation of the nucleotide sequence. D. The simplest (and most common) representation, with the abbreviations for the bases written in the conventional 5'→3' direction.

II. STRUCTURE OF DNA

DNA is a polymer of deoxyribonucleoside monophosphates (dNMPs) covalently linked by 3'→5'–phosphodiester bonds. With the exception of a few viruses that contain single-stranded (ss) DNA, DNA exists as a double-stranded (ds) molecule, in which the two strands wind around each other, forming a double helix. [Note: The sequence of the linked dNMPs is primary structure, whereas the double helix is secondary structure.] In eukaryotic cells, DNA is found associated with various types of proteins (known collectively as nucleoprotein) present in the nucleus, whereas in prokaryotes, the protein–DNA complex is present in a nonmembrane-bound region known as the nucleoid.

A. 3'→5'-Phosphodiester bonds

Phosphodiester bonds join the 3'-hydroxyl group of the deoxypentose of one nucleotide to the 5'-hydroxyl group of the deoxypentose of an adjacent nucleotide through a phosphoryl group (Figure 29.2). The resulting long, unbranched chain has polarity, with both a 5'-end (the end with the free phosphate) and a 3'-end (the end with the free hydroxyl) that are not attached to other nucleotides. The bases located along the resulting deoxyribose–phosphate backbone are, by convention, always written in sequence from the 5'-end of the chain to the 3'-end. For example, the sequence of bases in the DNA shown in Figure 29.2D (5'-TACG-3') is read "thymine, adenine, cytosine, guanine." Phosphodiester linkages between nucleotides can be hydrolyzed enzymatically by a family of nucleases: deoxyribonucleases for DNA and ribonucleases for RNA, or cleaved hydrolytically by chemicals. [Note: Only RNA is cleaved by alkali.]

B. Double helix

In the double helix, the two chains are coiled around a common axis called the helical axis. The chains are paired in an antiparallel manner (that is, the 5'-end of one strand is paired with the 3'-end of the other strand) as shown in Figure 29.3. In the DNA helix, the hydrophilic deoxyribose–phosphate backbone of each chain is on the outside of the molecule, whereas the hydrophobic bases are stacked inside. The overall structure resembles a twisted ladder. The spatial relationship between the two strands in the helix creates a major (wide) groove and a minor (narrow) groove. These grooves provide access for the binding of regulatory proteins to their specific recognition sequences along the DNA chain. [Note: Certain anticancer drugs, such as dactinomycin (actinomycin D), exert their cytotoxic effect by intercalating into the narrow groove of the DNA double helix, thereby interfering with DNA (and RNA) synthesis.]

1. **Base-pairing:** The bases of one strand of DNA are paired with the bases of the second strand, so that an adenine (A) is always paired with a thymine (T) and a cytosine (C) is always paired with a guanine (G). [Note: The base pairs are perpendicular to the helical axis (see Figure 29.3).] Therefore, one polynucleotide chain of the DNA double helix is always the complement of the other. Given the sequence of bases on one chain, the sequence of bases on the complementary chain can be determined (Figure 29.4). [Note: The specific base-pairing in DNA leads to the Chargaff rule, which states that in any sample of dsDNA, the amount of A equals the amount of T, the amount of G equals the amount of C, and the total amount of purines equals the total amount of pyrimidines.] The base pairs are held together by hydrogen bonds: two between A and T and three between G and C (Figure 29.5). These hydrogen bonds, plus the hydrophobic interactions between the stacked bases, stabilize the structure of the double helix.

2. **Separation of the two DNA strands in the double helix:** The two strands of the double helix separate when hydrogen bonds between the paired bases are disrupted. Disruption can occur in the laboratory if the pH of the DNA solution is altered so that the nucleotide bases ionize, or if the solution is heated. [Note: Phosphodiester bonds are not broken by such treatment.] When DNA is heated, the temperature at which one half of the helical structure is lost is defined as the melting temperature (T_m). The loss of helical structure in DNA, called denaturation, can be monitored by measuring its absorbance at 260 nm. [Note: ssDNA has a higher relative absorbance at this wavelength than does dsDNA.] Because there are three hydrogen bonds between G and C but only two between A and T, DNA that contains high concentrations of A and T denatures at a lower temperature than G- and C-rich

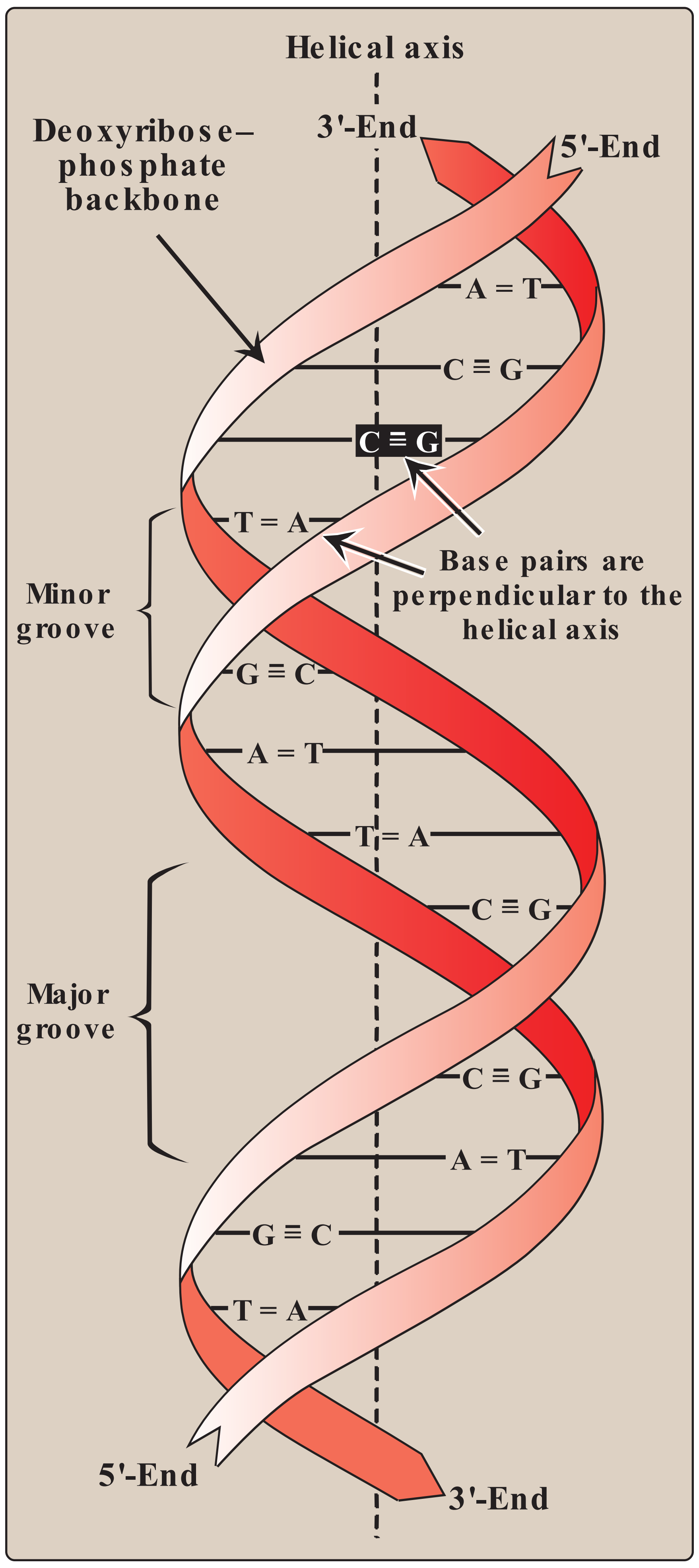


Figure 29.3
DNA double helix, illustrating some of its major structural features.

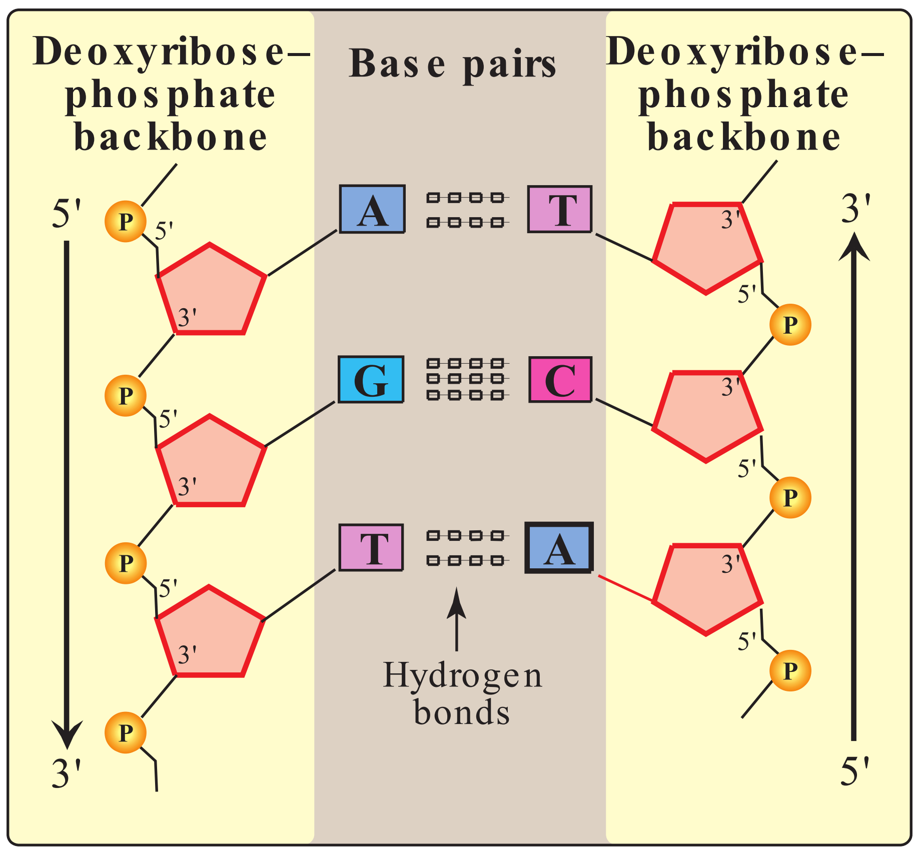

Deoxyribose–phosphate backbone
Base pairs
Deoxyribose–phosphate backbone
5'
3'
A
T
G
C
T
A
P
Hydrogen bonds
3'
5'

Figure 29.4
Two complementary DNA sequences. T= thymine; A = adenine; C = cytosine; G = guanine.

DNA (Figure 29.6). Under appropriate conditions, complementary DNA strands can reform the double helix by the process called renaturation (or reannealing). [Note: Separation of the two strands over short regions occurs during both DNA and RNA synthesis.]

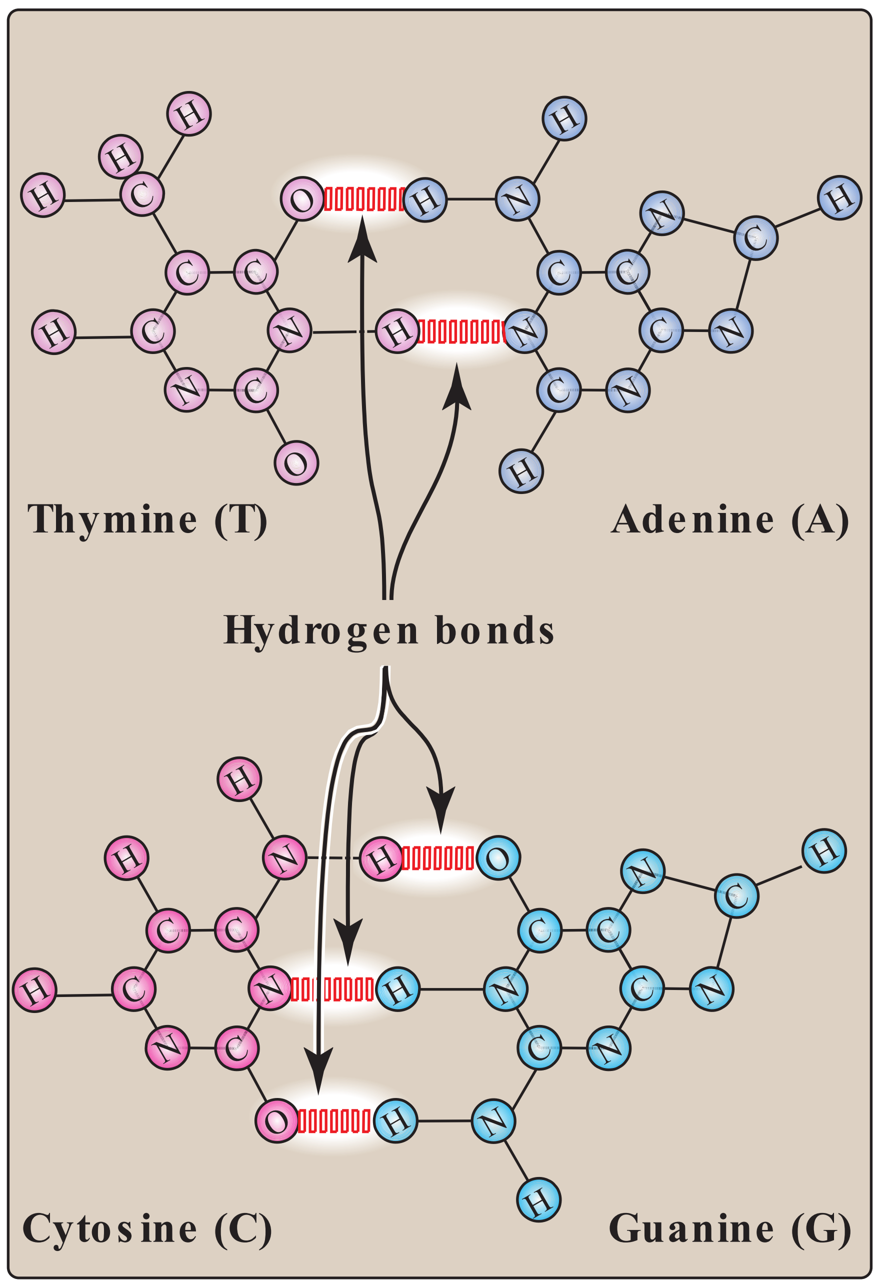


Figure 29.5
Hydrogen bonds between complementary bases.

3. **Structural forms of the double helix:** There are three major structural forms of DNA: the B form (described by Watson and Crick in 1953), the A form, and the Z form. The B form is a right-handed helix with 10 base pairs per 360° turn (or twist) of the helix, and with the planes of the bases perpendicular to the helical axis. Chromosomal DNA is thought to consist primarily of B-DNA (Figure 29.7 shows a space-filling model of B-DNA). The A form is produced by moderately dehydrating the B form. It is also a right-handed helix, but there are 11 base pairs per turn, and the planes of the base pairs are tilted 20° away from the perpendicular to the helical axis. The conformation found in DNA–RNA hybrids or RNA–RNA double-stranded regions is probably very close to the A form. Z-DNA is a left-handed helix that contains 12 base pairs per turn (see Figure 29.7). [Note: The deoxyribose–phosphate backbone "zigzags," hence, the name "Z"-DNA.] Stretches of Z-DNA can occur naturally in regions of DNA that have a sequence of alternating purines and pyrimidines (for example, poly GC). Transitions between the B and Z helical forms of DNA may play a role in regulating gene expression.

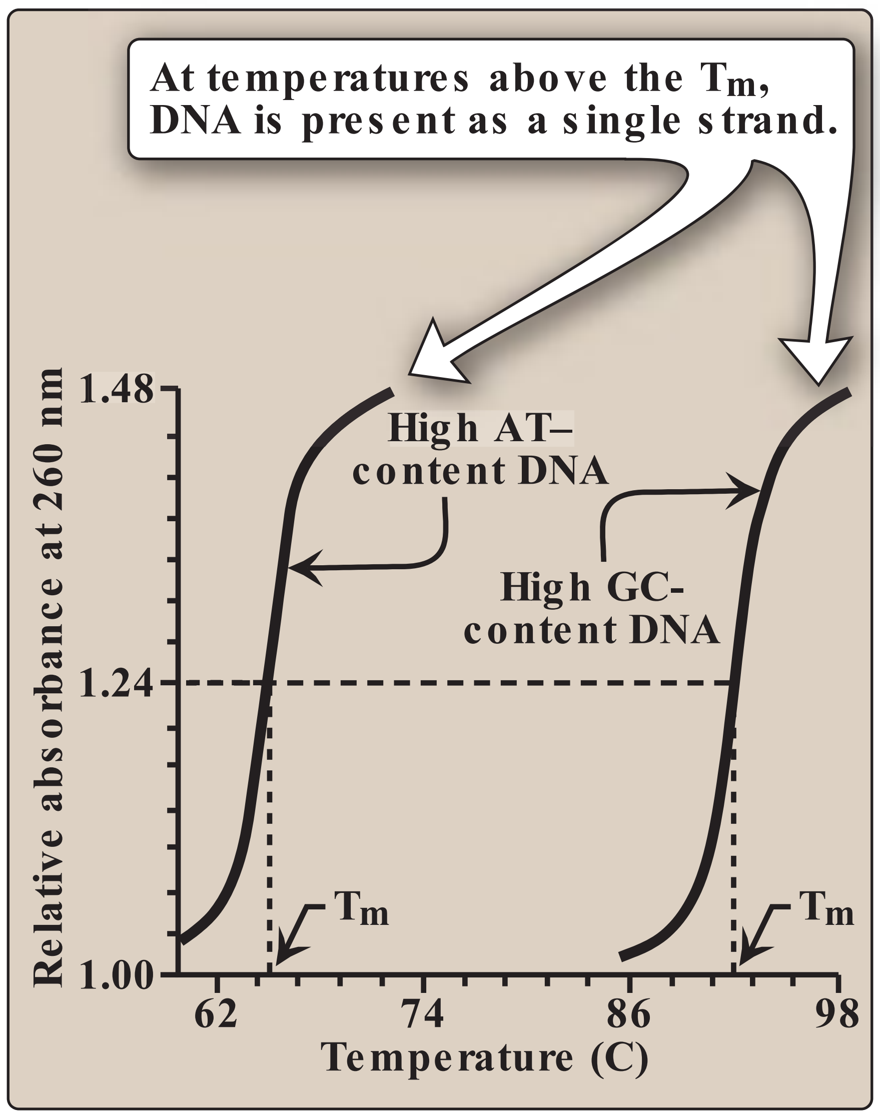


Figure 29.6
Melting temperatures (T_m) of DNA molecules with different nucleotide compositions.

C. Linear and circular DNA molecules

Each chromosome in the nucleus of a eukaryote contains one long, linear molecule of dsDNA, which is bound to a complex mixture of proteins (histone and nonhistone, see p. 409) to form chromatin. Eukaryotes have closed, circular, dsDNA molecules in their mitochondria, as do plant chloroplasts. A prokaryotic organism typically contains a single, circular, dsDNA molecule. [Note: Circular DNA is "supercoiled", that is, the double helix crosses over on itself one or more times. Supercoiling can result in overwinding (positive supercoiling) or underwinding (negative supercoiling) of DNA. Supercoiling, a type of tertiary structure, compacts DNA.] Each prokaryotic chromosome is associated with nonhistone proteins that help compact the DNA to form a nucleoid. In addition, most species of bacteria also contain small, circular, extrachromosomal DNA molecules called plasmids. Plasmid DNA carries genetic information, and undergoes replication that may or may not be synchronized to chromosomal division. [Note: The use of plasmids as vectors in recombinant DNA technology is described in Chapter 33.]

Plasmids may carry genes that convey antibiotic resistance to the host bacterium and may facilitate the transfer of genetic information from one bacterium to another.

III. STEPS IN PROKARYOTIC DNA SYNTHESIS

When the two strands of the DNA double helix are separated, each can serve as a template for the replication of a new complementary strand. This produces two daughter molecules, each of which contains two DNA strands with an antiparallel orientation (see Figure 29.3). This process is called semiconservative replication because, although the parental duplex is separated into two halves (and, therefore, is not "conserved" as an entity), each of the individual parental strands remains intact in one of the two new duplexes (Figure 29.8). The enzymes involved in the DNA replication process are template-directed polymerases that can synthesize the complementary sequence of each strand with extraordinary fidelity. The reactions described in this section were first known from studies of the bacterium Escherichia coli (E. coli), and the description given below refers to the process in prokaryotes. DNA synthesis in higher organisms is more complex but involves the same types of mechanisms. In either case, initiation of DNA replication commits the cell to continue the process until the entire genome has been replicated.

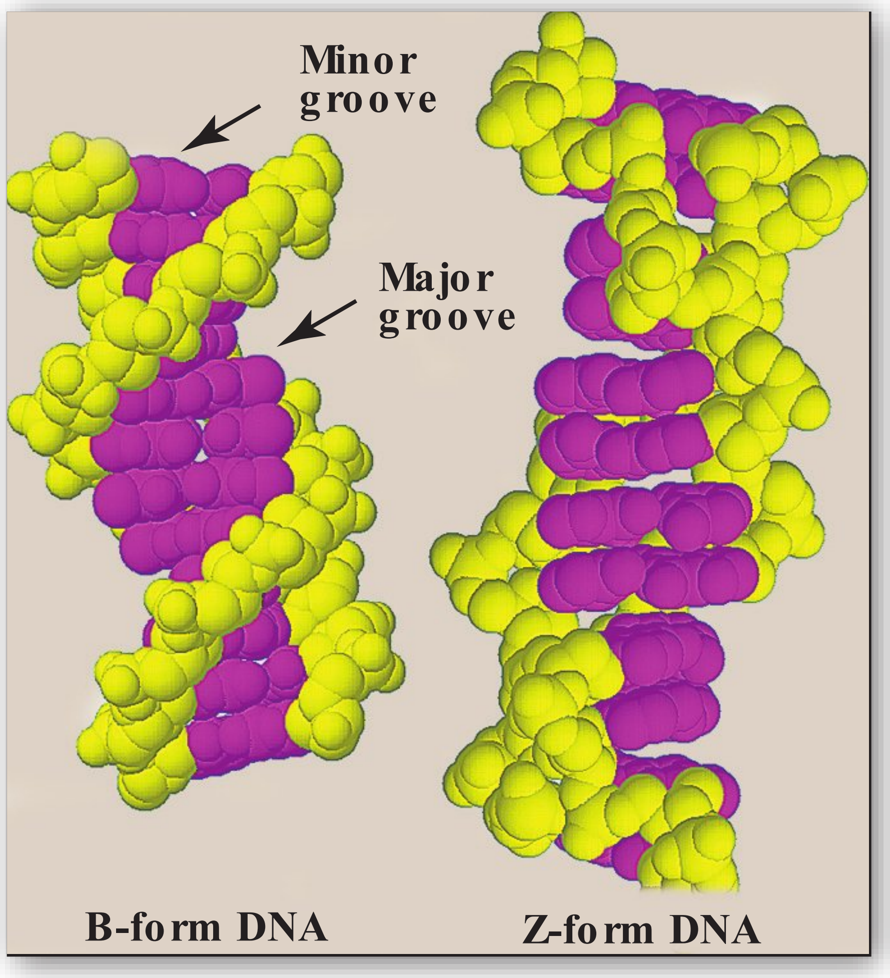


Figure 29.7
Structures of B-DNA and Z-DNA.

A. Separation of the two complementary DNA strands

In order for the two strands of the parental dsDNA to be replicated, they must first separate (or "melt") over a small region, because the polymerases use only ssDNA as a template. In prokaryotic organisms, DNA replication begins at a single, unique nucleotide sequence, a site called the origin of replication, or ori (Figure 29.9A). [Note: This sequence is referred to as a consensus sequence, because the order of nucleotides is essentially the same at each site.] The ori includes short, AT-rich segments that facilitate melting. In eukaryotes, replication begins at multiple sites along the DNA helix (Figure 29.9B). Having multiple origins of replication provides a mechanism for rapidly replicating the great length of eukaryotic DNA molecules.

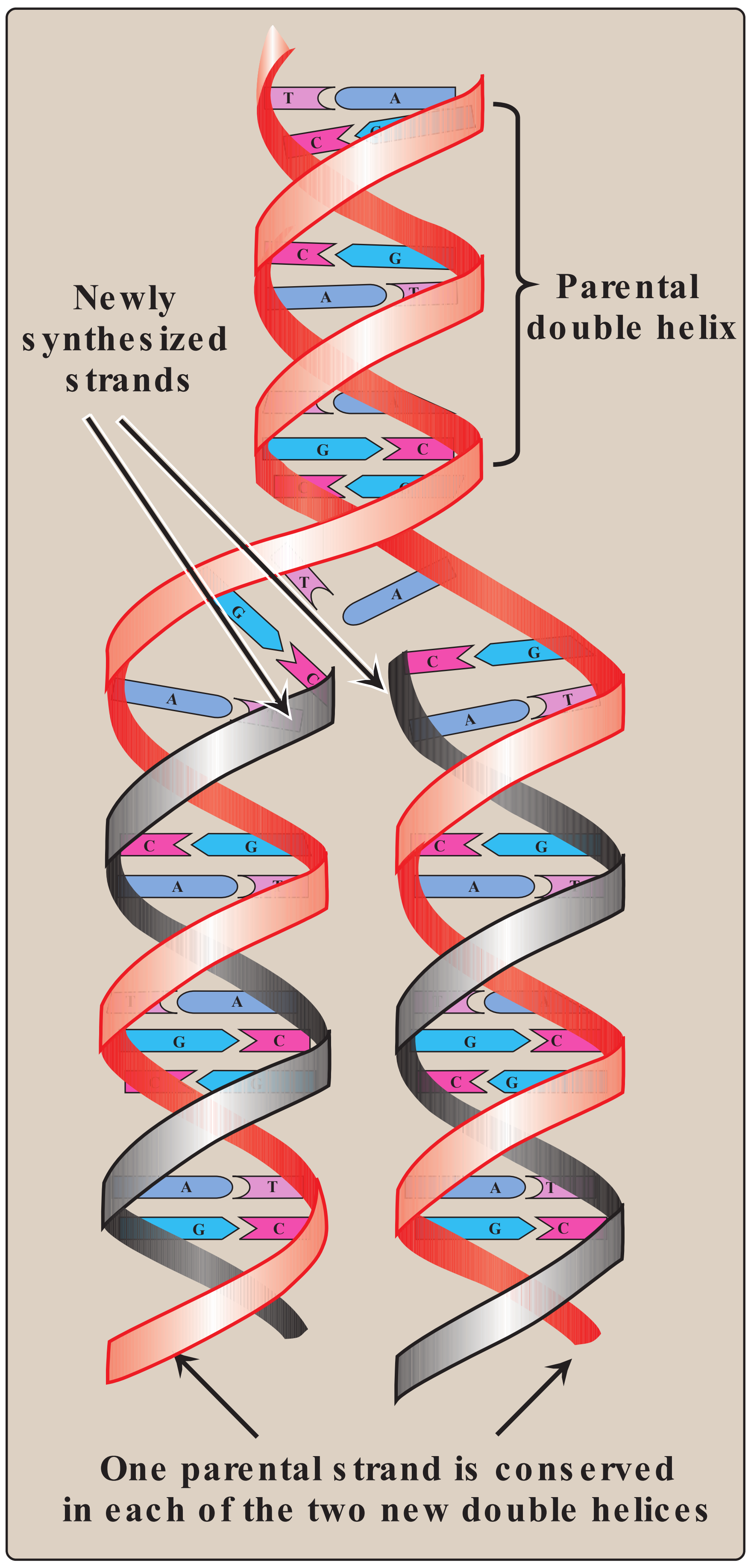

Parental double helix
Newly synthesized strands
One parental strand is conserved in each of the two new double helices

Figure 29.8
Semiconservative replication of DNA. T= thymine; A = adenine; C = cytosine; G = guanine.

B. Formation of the replication fork

As the two strands unwind and separate, synthesis occurs at two replication forks that move away from the origin in opposite directions (bidirectionally), generating a replication bubble (see Figure 29.9). [Note: The term "replication fork" derives from the Y-shaped structure in which the tines of the fork represent the separated strands (Figure 29.10).]

1. **Proteins required for DNA strand separation:** Initiation of DNA replication requires the recognition of the origin by a group of proteins that form the prepriming complex. These proteins are responsible for maintaining the separation of the parental strands, and for unwinding the double helix ahead of the advancing replication fork. These proteins include the following.

   a. **DnaA protein:** DnaA protein binds to specific nucleotide sequences (DnaA boxes) within the origin of replication, causing the short, tandemly arranged (one after the other) AT-rich regions in the origin to melt. Melting is adenosine triphosphate (ATP) dependent, and results in strand separation with the formation of localized regions of ssDNA.

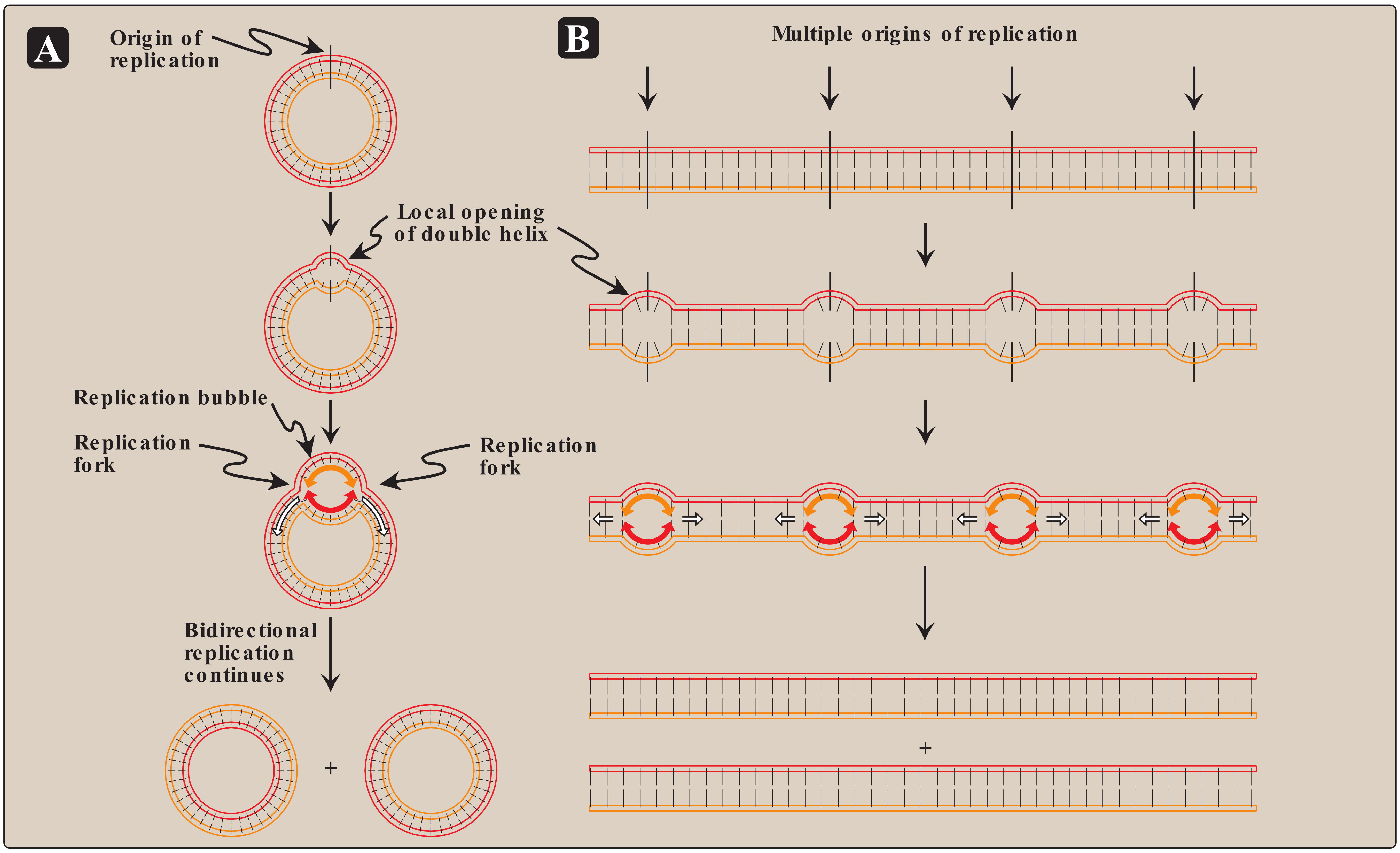

A
Origin of replication
Local opening of double helix
Replication bubble
Replication fork
Replication fork
Bidirectional replication continues
+
B
Multiple origins of replication
+

Figure 29.9
Replication of DNA: origins and replication forks. A. Small prokaryotic circular DNA. B. Very long eukaryotic DNA.

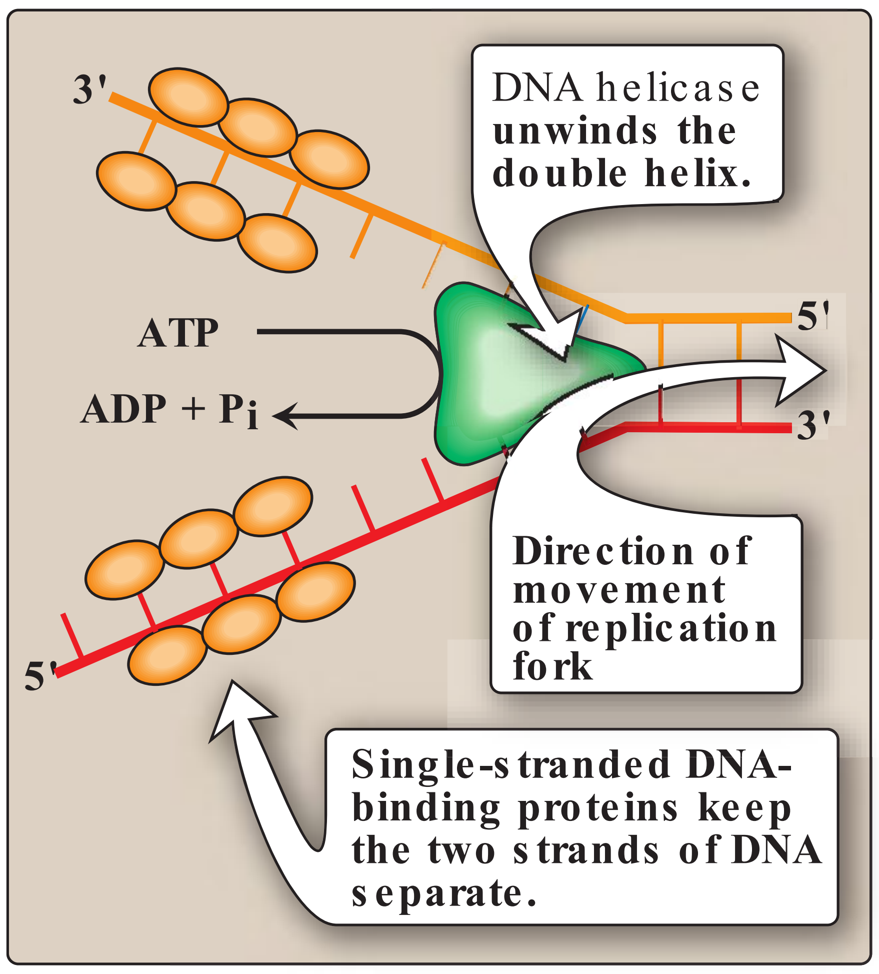

3'
DNA helicase unwinds the double helix.
5'
ATP
ADP + Pi
3'
Direction of movement of replication fork
5'
Single-stranded DNA-binding proteins keep the two strands of DNA separate.

Figure 29.10
Proteins responsible for maintaining the separation of the parental strands and unwinding the double helix ahead of the advancing replication fork (≻). ADP = adenosine diphosphate; P_i = inorganic phosphate.

b. **DNA helicases:** These enzymes bind to ssDNA near the replication fork and then move into the neighboring double-stranded region, forcing the strands apart (in effect, unwinding the double helix). Helicases require energy provided by ATP (see Figure 29.10). Unwinding at the replication fork causes supercoiling in other regions of the DNA molecule. [Note: DnaB is the principal helicase of replication in E. coli. Its binding to DNA requires DnaC.]

c. **Single-stranded DNA-binding protein:** This protein binds to the ssDNA generated by helicases (see Figure 29.10). Binding is cooperative (that is, the binding of one molecule of single-stranded binding [SSB] protein makes it easier for additional molecules of SSB protein to bind tightly to the DNA strand). The SSB proteins are not enzymes, but rather serve to shift the equilibrium between dsDNA and ssDNA in the direction of the single-stranded forms. These proteins not only keep the two strands of DNA separated in the area of the replication origin, thus providing the single-stranded template required by polymerases, but also protect the DNA from nucleases that degrade ssDNA.

2. **Solving the problem of supercoils:** As the two strands of the double helix are separated, a problem is encountered, namely, the appearance of positive supercoils in the region of DNA ahead

of the replication fork as a result of overwinding (Figure 29.11), and negative supercoils in the region behind the fork. The accumulating positive supercoils interfere with further unwinding of the double helix. [Note: Supercoiling can be demonstrated by tightly grasping one end of a helical telephone cord while twisting the other end. If the cord is twisted in the direction of tightening the coils, the cord will wrap around itself in space to form positive supercoils. If the cord is twisted in the direction of loosening the coils, the cord will wrap around itself in the opposite direction to form negative supercoils.] To solve this problem, there is a group of enzymes called DNA topoisomerases, which are responsible for removing supercoils in the helix by transiently cleaving one or both of the DNA strands.

a. Type I DNA topoisomerases: These enzymes reversibly cleave one strand of the double helix. They have both strand-cutting and strand-resealing activities. They do not require ATP, but rather appear to store the energy from the phosphodiester bond they cleave, reusing the energy to reseal the strand (Figure 29.12). Each time a transient "nick" is created in one DNA strand, the intact DNA strand is passed through the break before it is resealed, thus relieving ("relaxing") accumulated supercoils. Type I topoisomerases relax negative supercoils (that is, those that contain fewer turns of the helix than relaxed DNA) in E. coli, and both negative and positive supercoils (that is, those that contain fewer or more turns of the helix than relaxed DNA) in many prokaryotic cells (but not E. coli) and in eukaryotic cells.

b. Type II DNA topoisomerases: These enzymes bind tightly to the DNA double helix and make transient breaks in both strands. The enzyme then causes a second stretch of the DNA double helix to pass through the break and, finally, reseals the break (Figure 29.13). As a result, both negative and positive supercoils can be relieved by this ATP-requiring process. DNA gyrase, a type II topoisomerase found in bacteria and plants, has the unusual property of being able to introduce negative supercoils into circular DNA using energy from the hydrolysis of ATP. This facilitates the replication of DNA because the negative supercoils neutralize the positive supercoils introduced during opening of the double helix. It also aids in the transient strand separation required during transcription (see p. 420).

Anticancer agents, such as the camptothecins, target human type I topoisomerases, whereas etoposide targets human type II topoisomerases. Bacterial DNA gyrase is a unique target of a group of antimicrobial agents called fluoroquinolones (for example, ciprofloxacin).

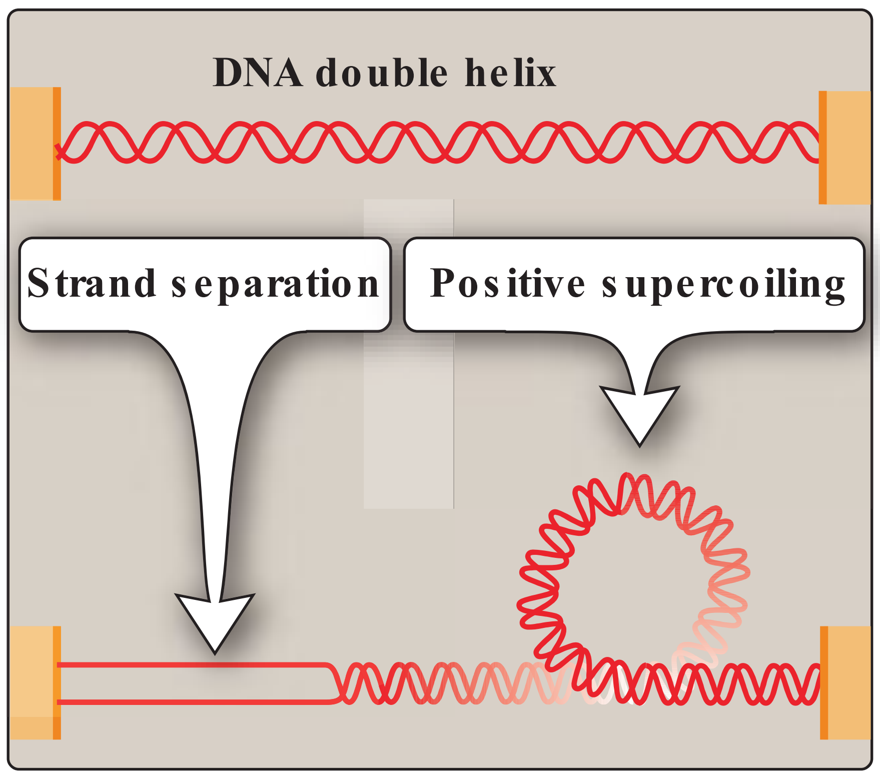


Figure 29.11
Positive supercoiling resulting from DNA strand separation.

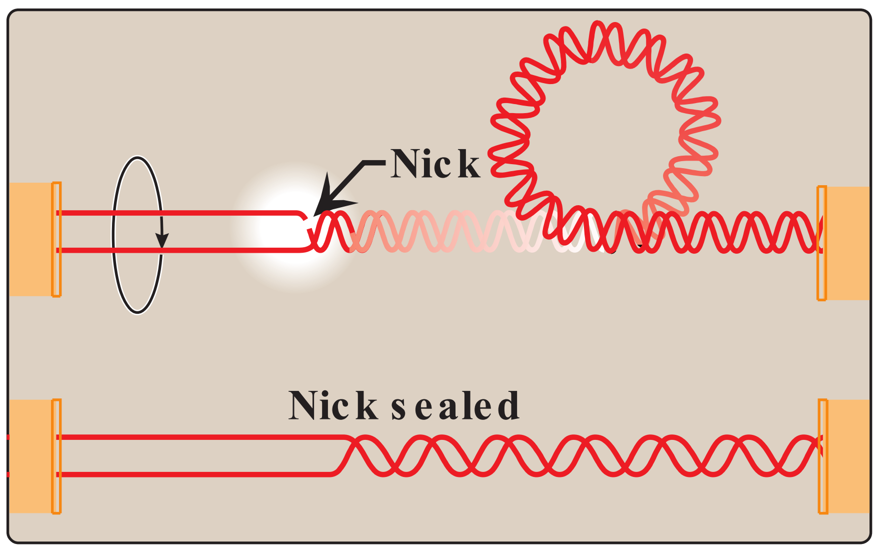


Figure 29.12
Action of type I DNA topoisomerases.

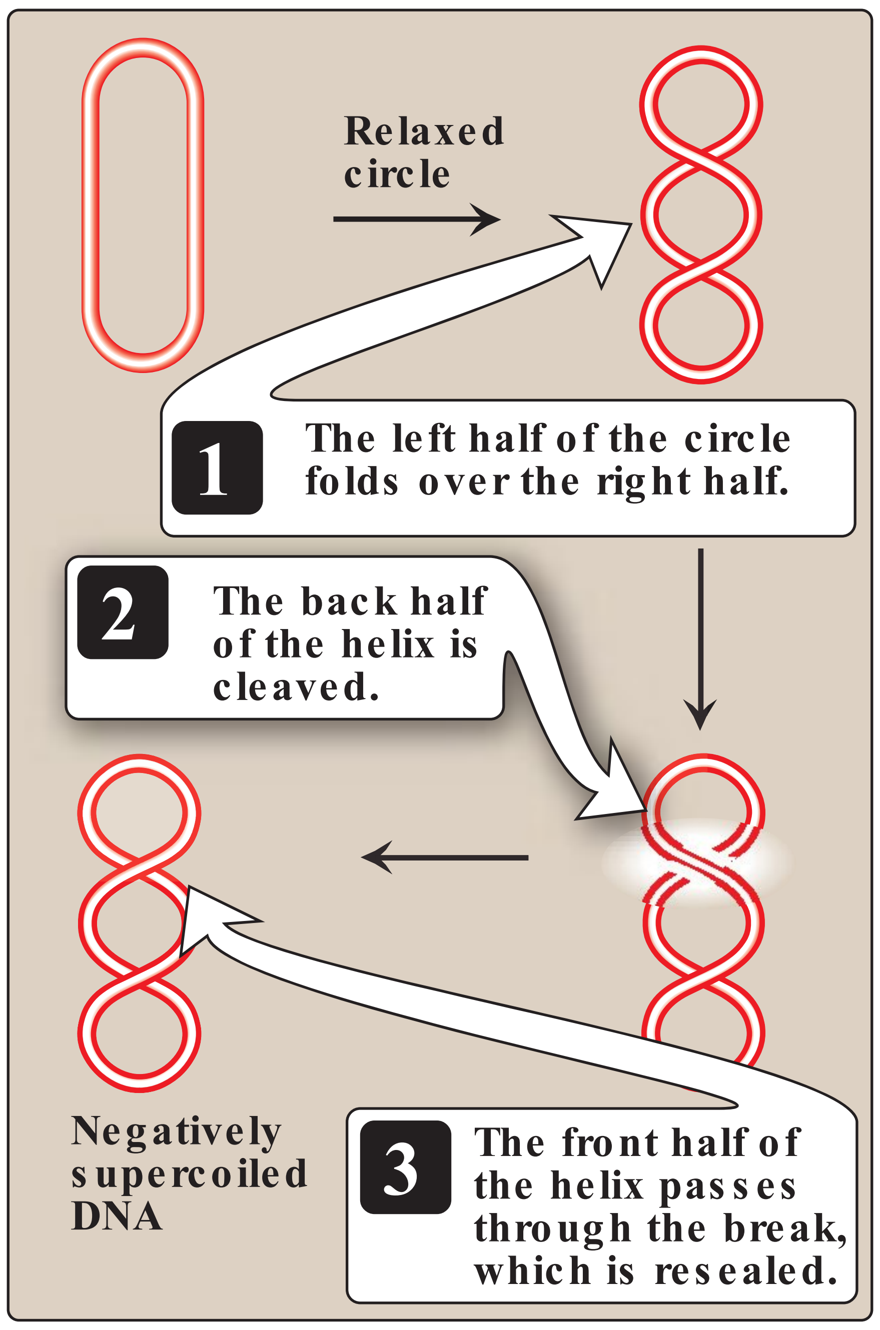

Relaxed circle
1 The left half of the circle folds over the right half.
2 The back half of the helix is cleaved.
Negatively supercoiled DNA
3 The front half of the helix passes through the break, which is resealed.

Figure 29.13
Action of type II DNA topoisomerase.

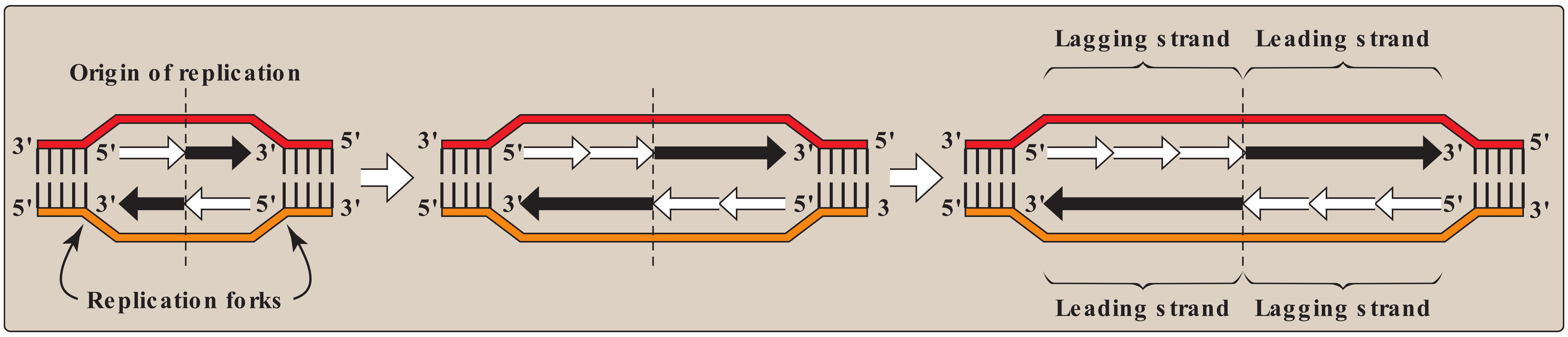

Origin of replication
Replication forks
Lagging strand
Leading strand
Leading strand
Lagging strand
3'
5'
3'
5'

Figure 29.14
Discontinuous synthesis of DNA.

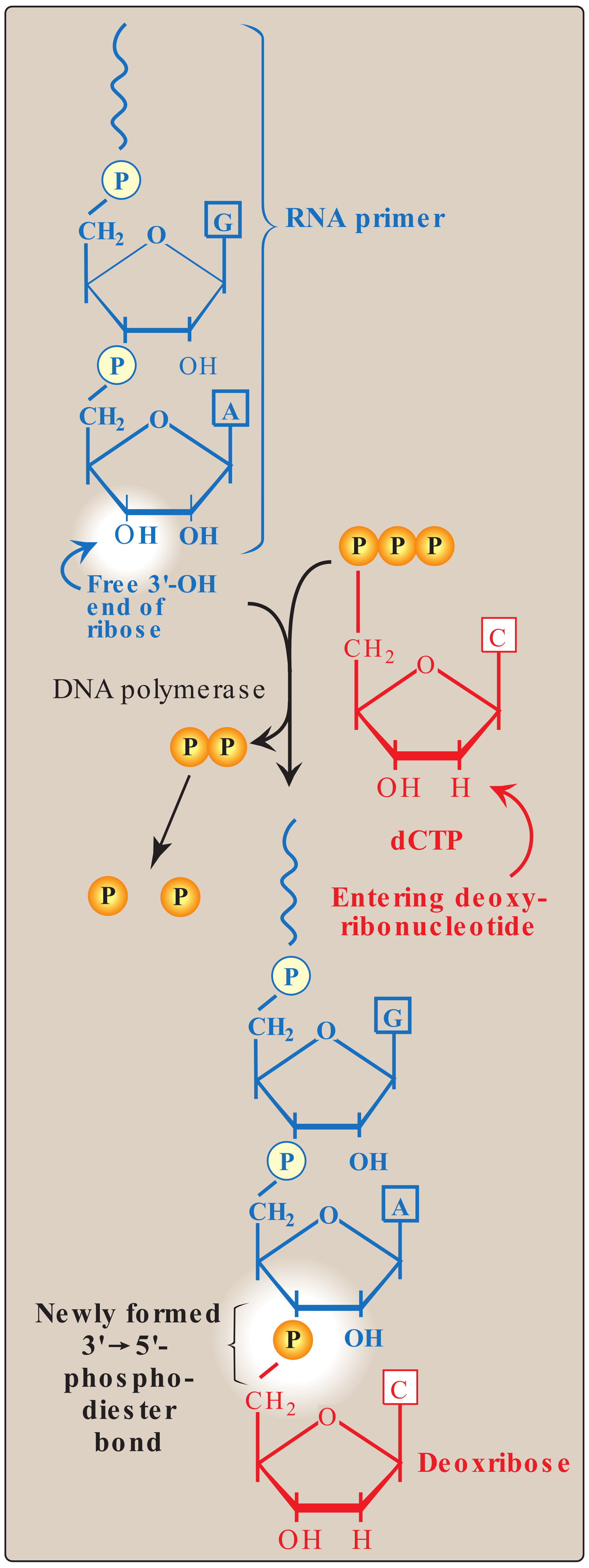

RNA primer
P
CH₂
O
G
OH
A
OH
Free 3'-OH end of ribose
DNA polymerase
P P P
P P
P P
C
H
dCTP
Entering deoxyribonucleotide
Newly formed 3'→5'-phosphodiester bond
Deoxribose

Figure 29.15
Use of an RNA primer to initiate DNA synthesis. P = phosphate.

C. Direction of DNA replication

The DNA polymerases responsible for copying the DNA templates are only able to "read" the parental nucleotide sequences in the 3'→5' direction, and they synthesize the new DNA strands only in the 5'→3' (antiparallel) direction. Therefore, beginning with one parental double helix, the two newly synthesized stretches of nucleotide chains must grow in opposite directions, one in the 5'→3' direction toward the replication fork and one in the 5'→3' direction away from the replication fork (Figure 29.14). This feat is accomplished by a slightly different mechanism on each strand.

1. **Leading strand:** The strand that is being copied in the direction of the advancing replication fork is called the leading strand and is synthesized continuously.

2. **Lagging strand:** The strand that is being copied in the direction away from the replication fork is synthesized discontinuously, with small fragments of DNA being copied near the replication fork. These short stretches of discontinuous DNA, termed Okazaki fragments, are eventually joined (ligated) to become a single, continuous strand. The new strand of DNA produced by this mechanism is termed the lagging strand.

D. RNA primer

DNA polymerases cannot initiate synthesis of a complementary strand of DNA on a totally single-stranded template. Rather, they require an RNA primer, which is a short, double-stranded region consisting of RNA base-paired to the DNA template, with a free hydroxyl group on the 3'-end of the RNA strand (Figure 29.15). This hydroxyl group serves as the first acceptor of a deoxynucleotide by action of a DNA polymerase. [Note: Recall that glycogen synthase also requires a primer (see p. 126).]

1. **Primase:** A specific RNA polymerase, called primase (DnaG), synthesizes the short stretches of RNA (approximately ten nucleotides long) that are complementary and antiparallel to the DNA template. In the resulting hybrid duplex, the U (uracil) in RNA pairs with A in DNA. As shown in Figure 29.16, these short RNA sequences are constantly being synthesized at the replication fork on the lagging strand, but only one RNA sequence at the origin of replication is required on the leading strand. The substrates

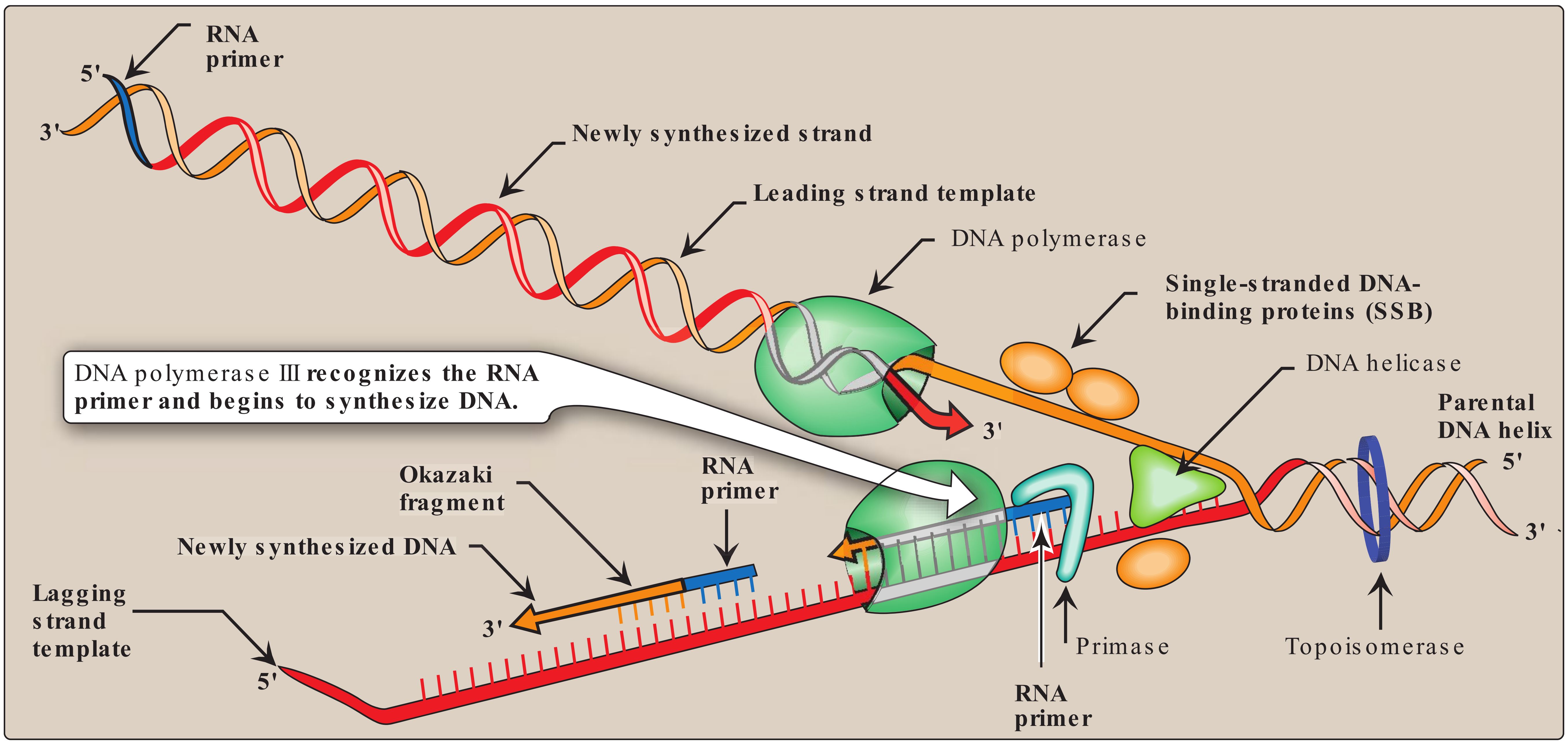

RNA primer
5'
3'
Newly synthesized strand
Leading strand template
DNA polymerase
Single-stranded DNA-binding proteins (SSB)
DNA helicase
Parental DNA helix
5'
3'
3'
DNA polymerase III recognizes the RNA primer and begins to synthesize DNA.
Okazaki fragment
RNA primer
Newly synthesized DNA
Lagging strand template
3'
5'
Primase
Topoisomerase
RNA primer

Figure 29.16
Elongation of the leading and lagging strands. [Note: The DNA sliding clamp is not shown.]

for this process are 5'-ribonucleoside triphosphates, and pyrophosphate is released as each ribonucleoside monophosphate is added through formation of a 3'→5' phosphodiester bond. [Note: The RNA primer is later removed as described on p. 405.]

2. **Primosome:** The addition of primase converts the prepriming complex of proteins required for DNA strand separation (see p. 399) to a primosome. The primosome makes the RNA primer required for leading strand synthesis and initiates Okazaki fragment formation in lagging strand synthesis. As with DNA synthesis, the direction of synthesis of the primer is 5'→3'.

E. Chain elongation

Prokaryotic (and eukaryotic) DNA polymerases (DNA pols) elongate a new DNA strand by adding deoxyribonucleotides, one at a time, to the 3'-end of the growing chain (see Figure 29.16). The sequence of nucleotides that are added is dictated by the base sequence of the template strand with which the incoming nucleotides are paired.

1. **DNA polymerase III:** DNA chain elongation is catalyzed by the multisubunit enzyme, DNA polymerase III (DNA pol III). Using the 3'-hydroxyl group of the RNA primer as the acceptor of the first deoxyribonucleotide, DNA pol III begins to add nucleotides along the single-stranded template that specifies the sequence of bases in the newly synthesized chain. DNA pol III is a highly "processive" enzyme (that is, it remains bound to the template strand as it moves along and does not diffuse away and then rebind before adding each new nucleotide). The processivity of DNA pol III is the result of its β subunit forming a ring that encircles and moves along the template strand of the DNA, thus serving as a sliding

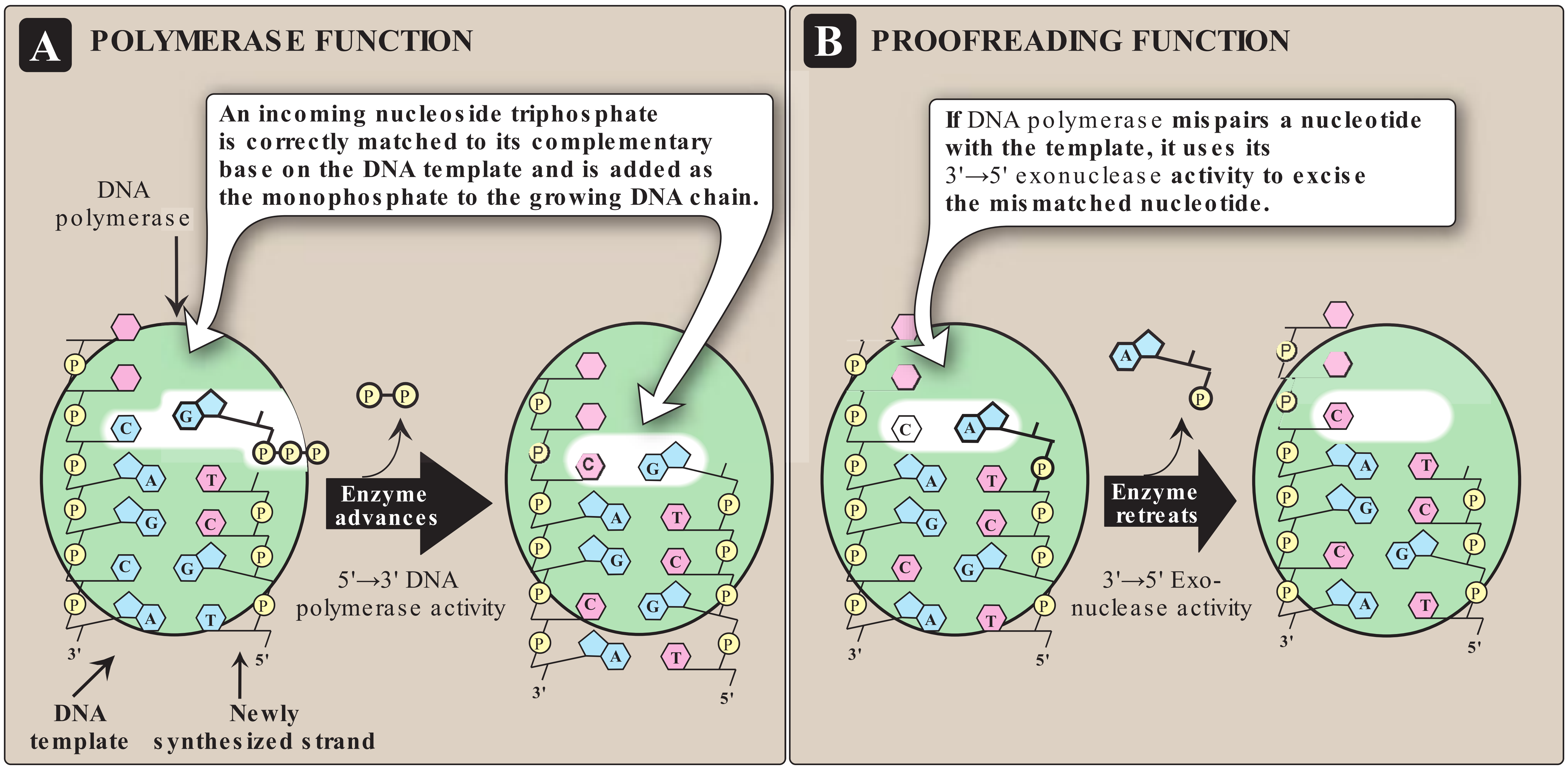

A
POLYMERASE FUNCTION
An incoming nucleoside triphosphate is correctly matched to its complementary base on the DNA template and is added as the monophosphate to the growing DNA chain.
DNA polymerase
Enzyme advances
5'→3' DNA polymerase activity
3'
5'
DNA template
Newly synthesized strand
B
PROOFREADING FUNCTION
If DNA polymerase mispairs a nucleotide with the template, it uses its 3'→5' exonuclease activity to excise the mismatched nucleotide.
Enzyme retreats
3'→5' Exonuclease activity

Figure 29.17
3'→5' Exonuclease activity enables DNA polymerase III to "proofread" the newly synthesized DNA strand.

DNA clamp. [Note: Clamp formation is facilitated by a protein complex, the clamp loader, and ATP hydrolysis.] The new strand grows in the 5'→3' direction, antiparallel to the parental strand (see Figure 29.16). The nucleotide substrates are 5'-deoxyribonucleoside triphosphates. Pyrophosphate (PP_i) is released when each new deoxynucleoside monophosphate is added to the growing chain (see Figure 29.15). Hydrolysis of PP_i to $2P_i$ means that a total of two high-energy bonds are used to drive the addition of each deoxynucleotide.

The production of PP_i with subsequent hydrolysis to $2P_i$, as seen in DNA replication, is a common theme in biochemistry. Removal of the PP_i product drives a reaction in the forward direction, making it essentially irreversible.

All four substrates (deoxyadenosine triphosphate [dATP], deoxythymidine triphosphate [dTTP], deoxycytidine triphosphate [dCTP], and deoxyguanosine triphosphate [dGTP]) must be present for DNA elongation to occur. If one of the four is in short supply, DNA synthesis stops when that nucleotide is depleted.

2. **Proofreading of newly synthesized DNA:** It is highly important for the survival of an organism that the nucleotide sequence of DNA be replicated with as few errors as possible. Misreading of the template sequence could result in deleterious, perhaps lethal,

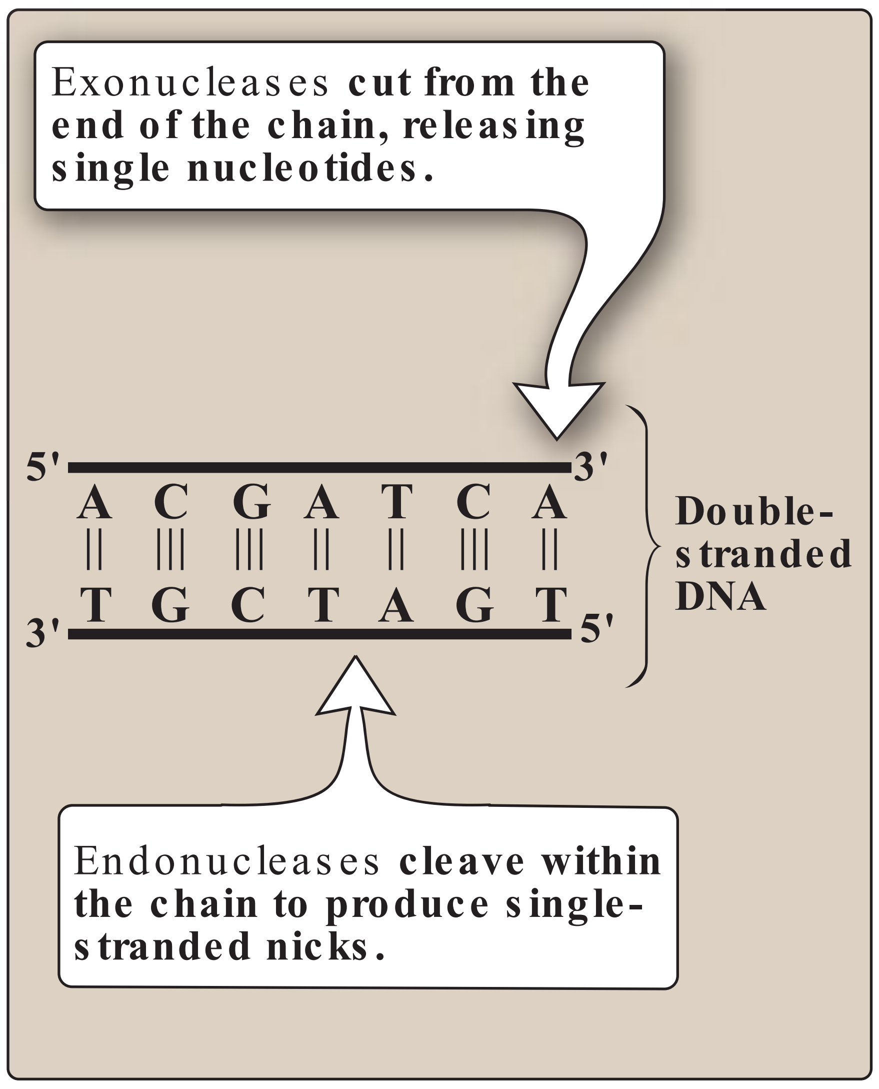
mutations. To ensure replication fidelity, DNA pol III has a "proofreading" activity (3'→5' exonuclease, Figure 29.17) in addition to its 5'→3' polymerase activity. As each nucleotide is added to the chain, DNA pol III checks to make certain the added nucleotide is, in fact, correctly matched to its complementary base on the template. If it is not, the 3'→5' exonuclease activity removes the error. [Note: The enzyme requires an improperly base-paired 3'-hydroxy terminus and, therefore, does not degrade correctly paired nucleotide sequences.] For example, if the template base is C and the enzyme mistakenly inserts an A instead of a G into the new chain, the 3'→5' exonuclease activity hydrolytically removes the misplaced nucleotide. The 5'→3' polymerase activity then replaces it with the correct nucleotide containing G (see Figure 29.17). [Note: The proofreading exonuclease activity requires movement in the 3'→5' direction, not 5'→3' like the polymerase activity. This is because the excision must be done in the reverse direction from that of synthesis.]

F. Excision of RNA primers and their replacement by DNA

DNA pol III continues to synthesize DNA on the lagging strand until it is blocked by proximity to an RNA primer. When this occurs, the RNA is excised and the gap filled by DNA pol I.

1. **5'→3' Exonuclease activity:** In addition to having the 5'→3' polymerase activity that synthesizes DNA and the 3'→5' exonuclease activity that proofreads the newly synthesized DNA chain like DNA pol III, DNA pol I also has a 5'→3' exonuclease activity that is able to hydrolytically remove the RNA primer. [Note: These activities are exonucleases because they remove nucleotides from the end of the DNA chain, rather than cleaving the chain internally as do the endonucleases (Figure 29.18).] First, DNA pol I locates the space (nick) between the 3'-end of the DNA newly synthesized by DNA pol III and the 5'-end of the adjacent RNA primer. Next, DNA pol I hydrolytically removes the RNA nucleotides "ahead" of itself, moving in the 5'→3' direction (5'→3' exonuclease activity). As it removes the RNA, DNA pol I replaces it with deoxyribonucleotides, synthesizing DNA in the 5'→3' direction (5'→3' polymerase activity). As it synthesizes the DNA, it also "proofreads" the new chain using its 3'→5' exonuclease activity to remove errors. This removal/synthesis/proofreading continues, one nucleotide at a time, until the RNA primer is totally degraded, and the gap is filled with DNA (Figure 29.19). [Note: DNA pol I uses its 5'→3' polymerase activity to fill in gaps generated during DNA repair (see p.410).]

2. **Comparison of 5'→3' and 3'→5' exonucleases:** The 5'→3' exonuclease activity of DNA pol I allows the polymerase, moving 5'→3', to hydrolytically remove one or more nucleotides at a time from the 5' end of the 20 to 30 nucleotide–long RNA primer. In contrast, the 3'→5' exonuclease activity of DNA pol I, as well as DNA pol II and III, allows these polymerases, moving 3'→5', to hydrolytically remove one misplaced nucleotide at a time from the 3' end of a growing DNA strand, increasing the fidelity of replication such that newly replicated DNA has one error per 10^7 nucleotides.



Exonucleases **cut from the end of the chain, releasing single nucleotides.**

Endonucleases **cleave within the chain to produce single-stranded nicks.**

Figure 29.18
Endonuclease versus exonuclease activity. [Note: Restriction endonucleases (see p. 465) cleave both strands.] T= thymine; A = adenine; C = cytosine; G = guanine.

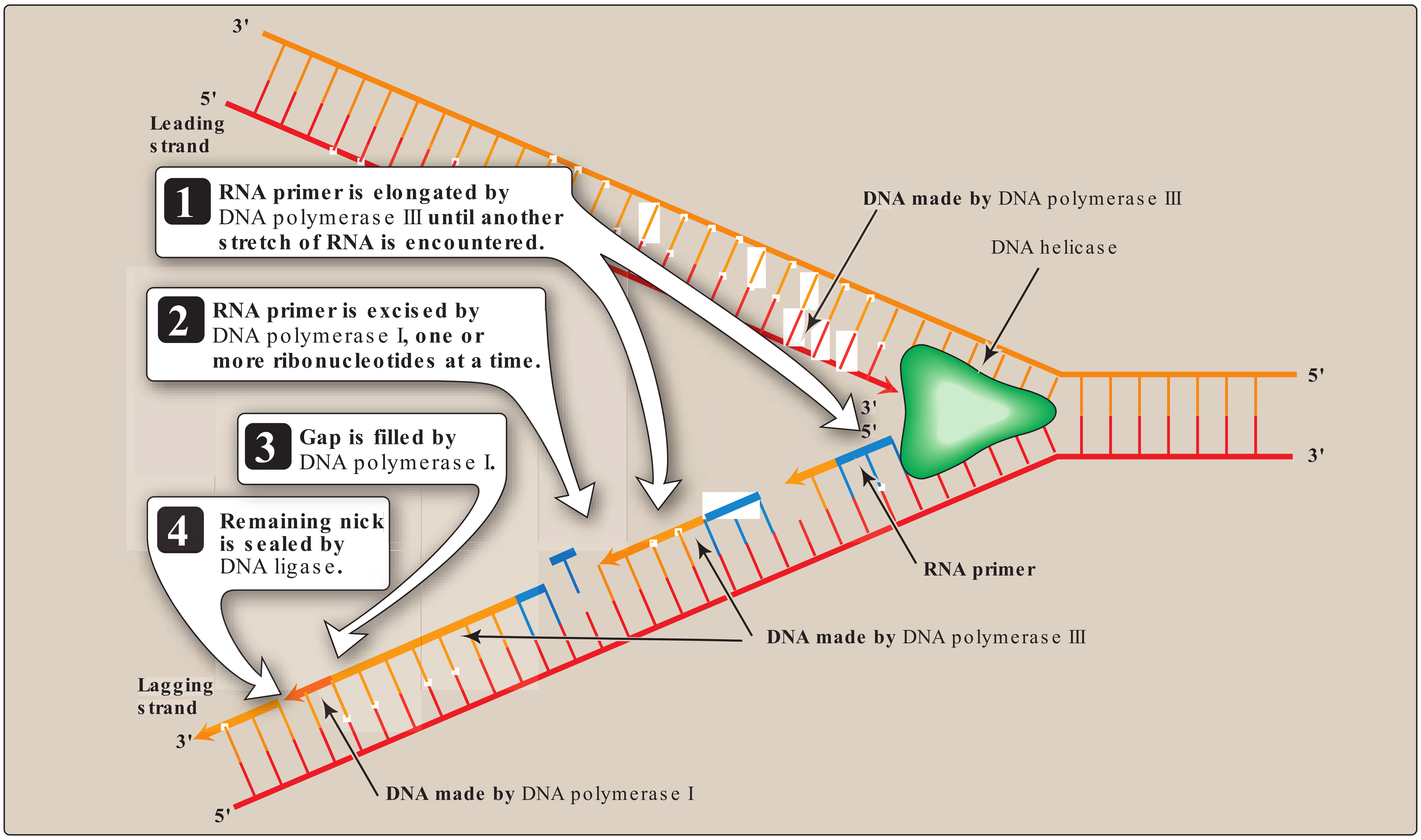

3'
5'
Leading strand
1 RNA primer is elongated by DNA polymerase III until another stretch of RNA is encountered.
2 RNA primer is excised by DNA polymerase I, one or more ribonucleotides at a time.
3 Gap is filled by DNA polymerase I.
4 Remaining nick is sealed by DNA ligase.
DNA made by DNA polymerase III
DNA helicase
5'
3'
3'
5'
RNA primer
DNA made by DNA polymerase III
Lagging strand
3'
DNA made by DNA polymerase I
5'

Figure 29.19
Removal of RNA primer and filling of the resulting "gaps" by DNA polymerase I.

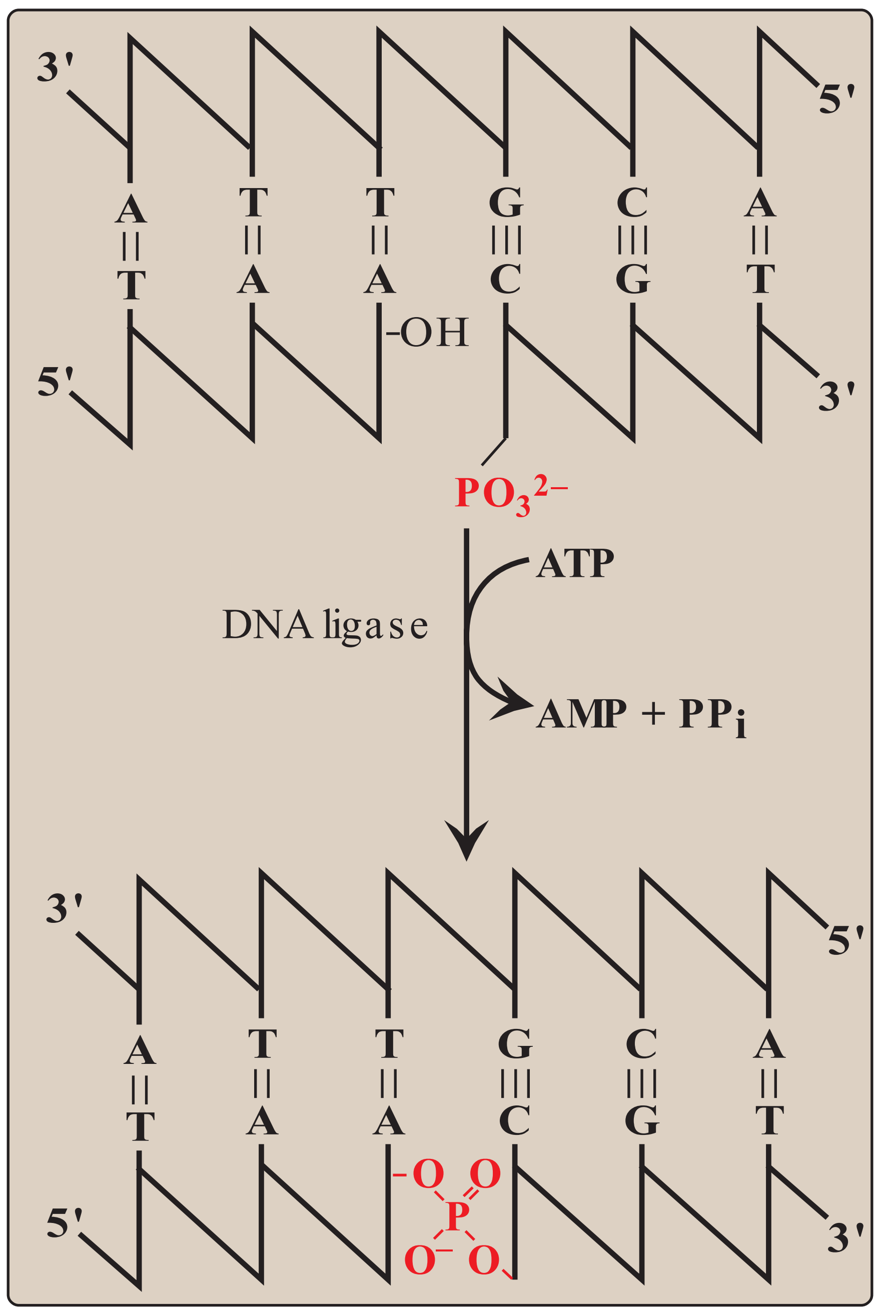

3'
5'
A T T G C A
T A A C G T
-OH
5'
3'
PO$_3^{2-}$
ATP
DNA ligase
AMP + PP$_i$
3'
5'
A T T G C A
T A A C G T
O O P O$^-$ O
5'
3'

Figure 29.20
Formation of a phosphodiester bond by DNA ligase. [Note: AMP is first linked to ligase, then to the 5' phosphate, and then released.]

G. DNA ligase

The final phosphodiester linkage between the 5'-phosphate group on the DNA chain synthesized by DNA pol III and the 3'-hydroxyl group on the chain made by DNA pol I is catalyzed by DNA ligase (Figure 29.20). The joining of these two stretches of DNA requires energy, which in most organisms is provided by the cleavage of ATP to AMP + PP$_i$.

H. Termination

Replication termination in E. coli is mediated by sequence-specific binding of the protein, Tus (terminus utilization substance) to replication termination sites (ter sites) on the DNA, stopping the movement of DNA polymerase.

IV. EUKARYOTIC DNA REPLICATION

The process of eukaryotic DNA replication closely follows that of prokaryotic DNA synthesis. Some differences, such as the multiple origins of replication in eukaryotic cells versus single origins of replication in prokaryotes, have already been noted. Eukaryotic single-stranded DNA-binding proteins and ATP-dependent DNA helicases have been identified, whose functions are analogous to those of the prokaryotic enzymes previously discussed. In contrast, RNA primers are removed by RNase H and flap endonuclease-1 (FEN1) rather than by a DNA polymerase (Figure 29.21).

A. Eukaryotic cell cycle

The events surrounding eukaryotic DNA replication and cell division (mitosis) are coordinated to produce the cell cycle (Figure 29.22). The period preceding replication is called the G_1 phase (Gap 1). DNA replication occurs during the S (synthesis) phase. Following DNA synthesis, there is another period (G_2 phase, or Gap 2) before mitosis (M). Cells that have stopped dividing, such as mature T lymphocytes, are said to have gone out of the cell cycle into the G_0 phase. Such quiescent cells can be stimulated to reenter the G_1 phase to resume division. [Note: The cell cycle is controlled at a series of "checkpoints" that prevent entry into the next phase of the cycle until the preceding phase has been completed. Two key classes of proteins that control the progress of a cell through the cell cycle are the cyclins and cyclin-dependent kinases (Cdks).]

FUNCTION	PROTEIN (s)
Origin recognition	ORC
Helicase activity	MCM
ssDNA protection	RPA
Primer synthesis	Pol a/primase
Sliding clamp	PCNA
Primer removal	RNase H, FEN 1

Figure 29.21
Proteins and their function in eukaryotic replication. ORC = origin recognition complex, MCM = mini-chromosome maintenance (complex), RPA = replication protein A, PCNA = proliferating cell nuclear antigen.

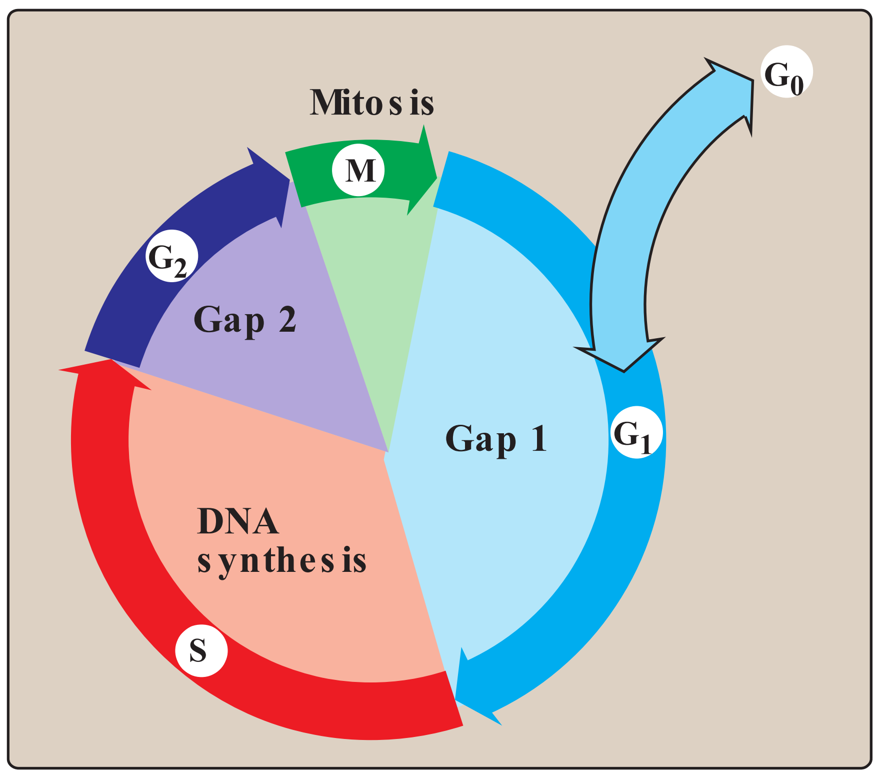


Figure 29.22
The eukaryotic cell cycle. [Note: Cells can leave the cell cycle and enter a reversible quiescent state called G_0.]

B. Eukaryotic DNA polymerases

At least five high-fidelity eukaryotic DNA polymerases (pol) have been identified and categorized on the basis of molecular weight, cellular location, sensitivity to inhibitors, and the templates or substrates on which they act. They are designated by Greek letters rather than by Roman numerals (Figure 29.23).

1. **Pol α:** Pol *α* is a multisubunit enzyme. One subunit has primase activity, which initiates strand synthesis on the leading strand and at the beginning of each Okazaki fragment on the lagging strand. The primase subunit synthesizes a short RNA primer that is extended by the 5'→3' polymerase activity of pol *α*, generating a short piece of DNA. [Note: Pol *α* is also referred to as pol *α*/primase.]
2. **Pol ε and pol d:** Pol *ε* is recruited to complete DNA synthesis on the leading strand, whereas pol *d* elongates the Okazaki fragments of the lagging strand, each using 3'→5' exonuclease activity to proofread the newly synthesized DNA. [Note: DNA pol *ε* associates with proliferating cell nuclear antigen (PCNA), a protein that serves as a sliding DNA clamp in much the same way the β subunit of DNA pol III does in E. coli, thus ensuring high processivity.]
3. **Pol β and pol g:** Pol *β* is involved in "gap filling" in DNA repair (see below). Pol *γ* replicates mitochondrial DNA.

POLY-MERASE	FUNCTION	PROOF-READING*
Pol **α** (alpha)	• **Contains primase** • **Initiates DNA synthesis**	–
Pol **β** (beta)	• **Repair**	–
Pol **γ** (gamma)	• **Replicates mitochondrial DNA**	+
Pol **δ** (delta)	• **Elongates Okazaki fragments of the lagging strand**	+
Pol **ε** (epsilon)	• **Elongates the leading strand**	+

Figure 29.23
Activities of eukaryotic DNA polymerase (pol) *3'→5' exonuclease activity.

C. Telomeres

Telomeres are complexes of noncoding DNA plus proteins (collectively known as shelterin) located at the ends of linear chromosomes. They maintain the structural integrity of the chromosome, preventing attack by nucleases, and allow repair systems to distinguish a true end from a break in dsDNA. In humans, telomeric DNA consists of several thousand tandem repeats of a noncoding hexameric sequence, AGGGTT, base-paired to a complementary region of Cs and As. The GT-rich strand is longer than its CA complement, leaving ssDNA a few hundred nucleotides in length at the 3'-end. The single-stranded region is thought to fold back on itself, forming a loop structure that is stabilized by protein.

1. **Telomere shortening:** Eukaryotic cells face a special problem in replicating the ends of their linear DNA molecules. Following

removal of the RNA primer from the extreme 5'-end of the lagging strand, there is no way to fill in the remaining gap with DNA. Consequently, in most normal human somatic cells, telomeres shorten with each successive cell division. Once telomeres are shortened beyond some critical length, the cell is no longer able to divide and is said to be senescent. In germ cells and other stem cells, as well as in cancer cells, telomeres do not shorten and the cells do not senesce. This is a result of the presence of a ribonucleoprotein, telomerase, which maintains telomeric length in these cells.

2. **Telomerase:** This complex contains a protein (Tert) that acts as a reverse transcriptase and a short piece of RNA (Terc) that acts as a template. The CA-rich RNA template base-pairs with the GT-rich, single-stranded 3'-end of telomeric DNA (Figure 29.24). The reverse transcriptase uses the RNA template to synthesize DNA in the usual 5'→3' direction, extending the already longer 3'-end. Telomerase then translocates to the newly synthesized end, and the process is repeated. Once the GT-rich strand has been lengthened, primase activity of DNA pol *α* can use it as a template to synthesize an RNA primer. The RNA primer is extended by DNA pol *α*, and then removed.

> Telomeres may be viewed as mitotic clocks in that their length in most cells is inversely related to the number of times the cells have divided. The study of telomeres provides insight into the biology of aging and cancer.

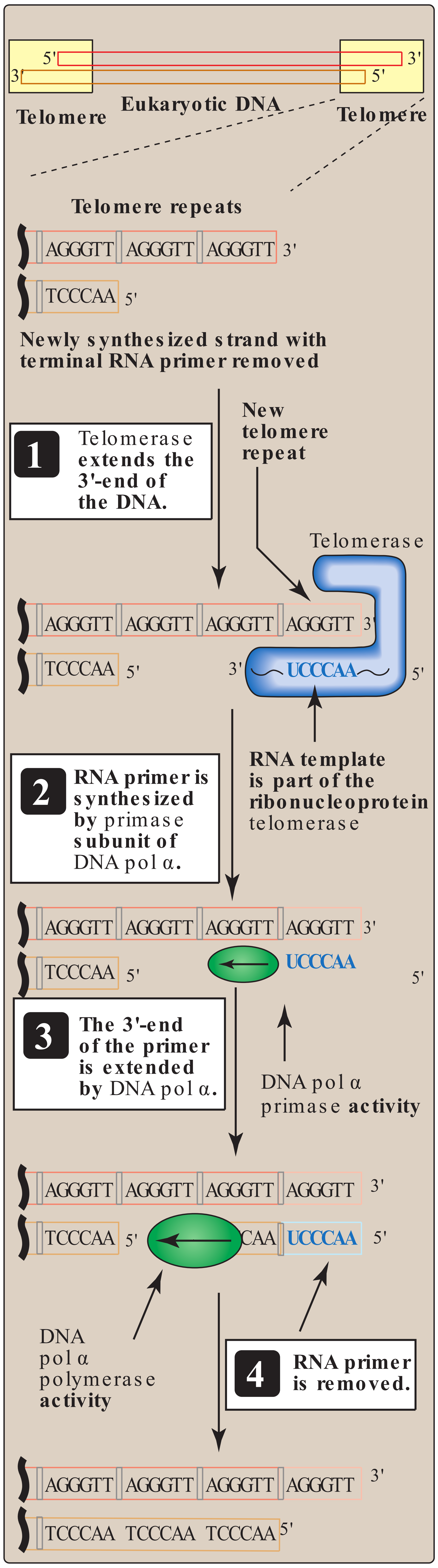

5'
3'
3'
5'
Telomere
Eukaryotic DNA
Telomere
Telomere repeats
AGGGTT AGGGTT AGGGTT 3'
TCCCAA 5'
Newly synthesized strand with terminal RNA primer removed
1 Telomerase extends the 3'-end of the DNA.
New telomere repeat
Telomerase
AGGGTT AGGGTT AGGGTT AGGGTT 3'
TCCCAA 5'
3' UCCCAA 5'
RNA template is part of the ribonucleoprotein telomerase
2 RNA primer is synthesized by primase subunit of DNA pol α.
AGGGTT AGGGTT AGGGTT AGGGTT 3'
TCCCAA 5'
UCCCAA
5'
3 The 3'-end of the primer is extended by DNA pol α.
DNA pol α primase activity
AGGGTT AGGGTT AGGGTT AGGGTT 3'
TCCCAA 5'
CAA UCCCAA 5'
DNA pol α polymerase activity
4 RNA primer is removed.
AGGGTT AGGGTT AGGGTT AGGGTT 3'
TCCCAA TCCCAA TCCCAA 5'

Figure 29.24
Mechanism of action of telomerase. T= thymine; A = adenine; C = cytosine; G = guanine; pol = polymerase.

D. Reverse transcriptases

As seen with telomerase, reverse transcriptases are RNA-directed DNA polymerases. A reverse transcriptase is involved in the replication of retroviruses, such as human immunodeficiency virus (HIV). These viruses carry their genome in the form of ssRNA molecules. Following infection of a host cell, the viral enzyme reverse transcriptase uses the viral RNA as a template for the 5'→3' synthesis of viral DNA, which then becomes integrated into host chromosomes. Reverse transcriptase activity is also seen with transposons, DNA elements that can move about the genome (see p. 461). In eukaryotes, such elements are transcribed to RNA, the RNA is used as a template for DNA synthesis by a reverse transcriptase encoded by the transposon, and the DNA is randomly inserted into the genome. [Note: Transposons that involve an RNA intermediate are called retrotransposons or retroposons.]

E. Inhibition of DNA synthesis by nucleoside analogs

DNA chain growth can be blocked by the incorporation of certain nucleoside analogs that have been modified on the sugar portion (Figure 29.25). For example, removal of the hydroxyl group from the 3'-carbon of the deoxyribose ring as in 2',3'-dideoxyinosine ([ddI] also known as didanosine), or conversion of the deoxyribose to another sugar, such as arabinose, prevents further chain elongation. By blocking DNA replication, these compounds slow the division of rapidly growing cells and viruses. Cytosine arabinoside (cytarabine, or araC) has been used in anticancer chemotherapy, whereas adenine arabinoside (vidarabine, or araA) is an antiviral agent. Substitution on the sugar moiety, as seen in zidovudine (AZT, ZDV), also terminates DNA chain elongation. [Note: These drugs are generally supplied as nucleosides, which are then converted to the active nucleotides by cellular kinases.

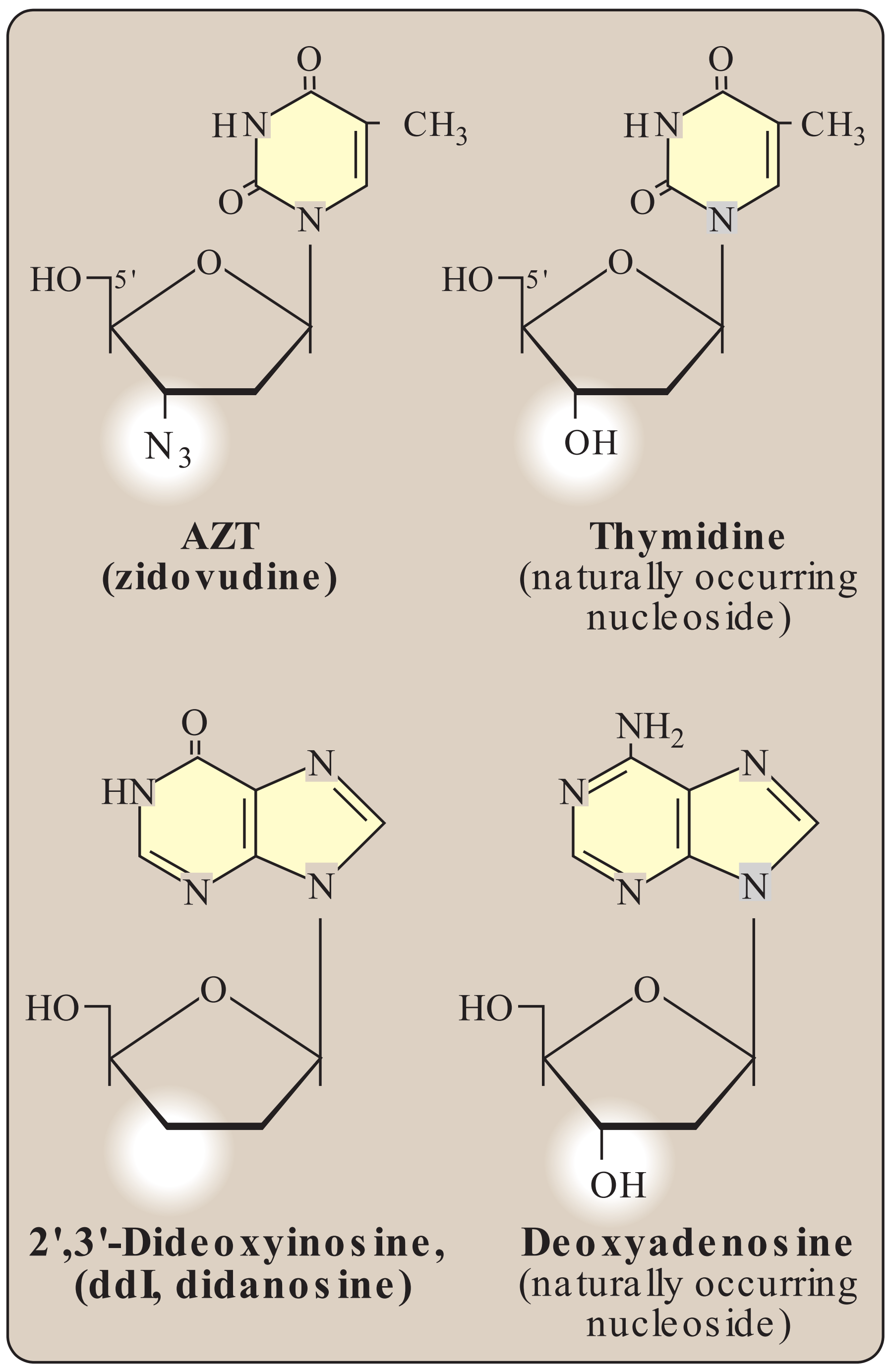


Figure 29.25
Examples of nucleoside analogs that lack a 3'-hydroxyl group. [Note: ddI is converted to its active form (ddATP).]

V. ORGANIZATION OF EUKARYOTIC DNA

A typical (diploid) human cell contains 46 chromosomes, whose total DNA is approximately 2 m long! It is difficult to imagine how such a large amount of genetic material can be effectively packaged into a volume the size of a cell nucleus so that it can be efficiently replicated, and its genetic information expressed. To do so requires the interaction of DNA with a large number of proteins, each of which performs a specific function in the ordered packaging of these long molecules of DNA. Eukaryotic DNA is associated with tightly bound basic proteins, called histones. These serve to order the DNA into fundamental structural units, called nucleosomes, which resemble beads on a string. Nucleosomes are further arranged into increasingly more complex structures that organize and condense the long DNA molecules into chromosomes that can be segregated during cell division. [Note: The complex of DNA and protein found inside the nuclei of eukaryotic cells is called chromatin.]

A. Histones and the formation of nucleosomes

There are five classes of histones, designated H1, H2A, H2B, H3, and H4. These small, evolutionally conserved proteins are positively charged at physiologic pH as a result of their high content of lysine and arginine. Because of their positive charge, they form ionic bonds with negatively charged DNA. Histones, along with ions such as Mg^{2+}, help neutralize the negatively charged DNA phosphate groups.

1. **Nucleosomes:** Two molecules each of H2A, H2B, H3, and H4 form the octameric core of the individual nucleosome "beads." Around this structural core, a segment of dsDNA is wound nearly twice, causing supercoiling (Figure 29.26). [Note: The N-terminal ends of these histones can be acetylated, methylated, or phosphorylated. These reversible covalent modifications influence how tightly the histones bind to the DNA, thereby affecting the expression of specific genes (see p. 422). Histone modification is an example of "epigenetics" or heritable changes in gene expression without alteration of the nucleotide sequence.] Neighboring nucleosomes are joined by "linker" DNA approximately 50 base pairs long. H1, of which there are several related species, is not found in the nucleosome core, but instead binds to the linker DNA chain between the nucleosome beads. H1 is the most tissue specific and species specific of the histones. It facilitates the packing of nucleosomes into more compact structures.

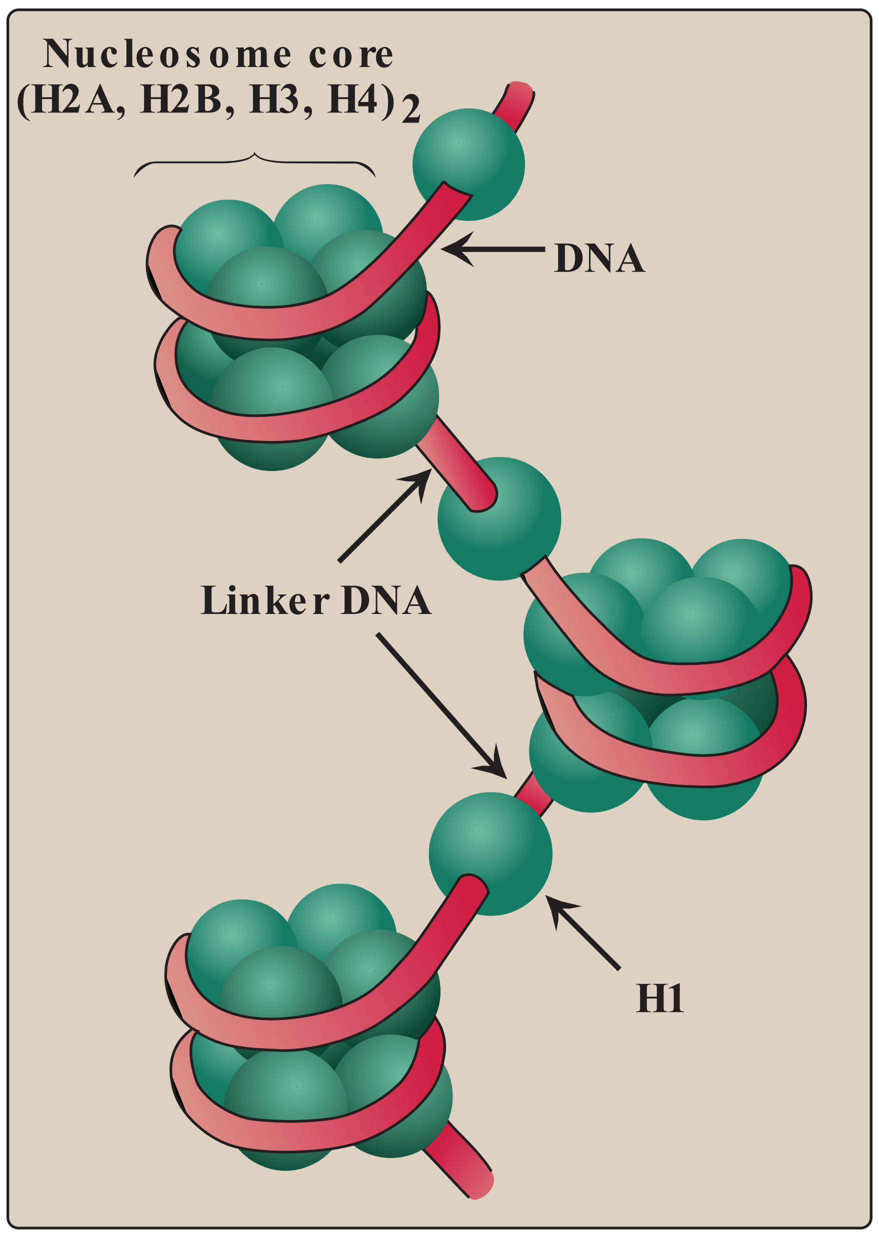


Figure 29.26
Organization of human DNA, illustrating the structure of nucleosomes. H = histone.

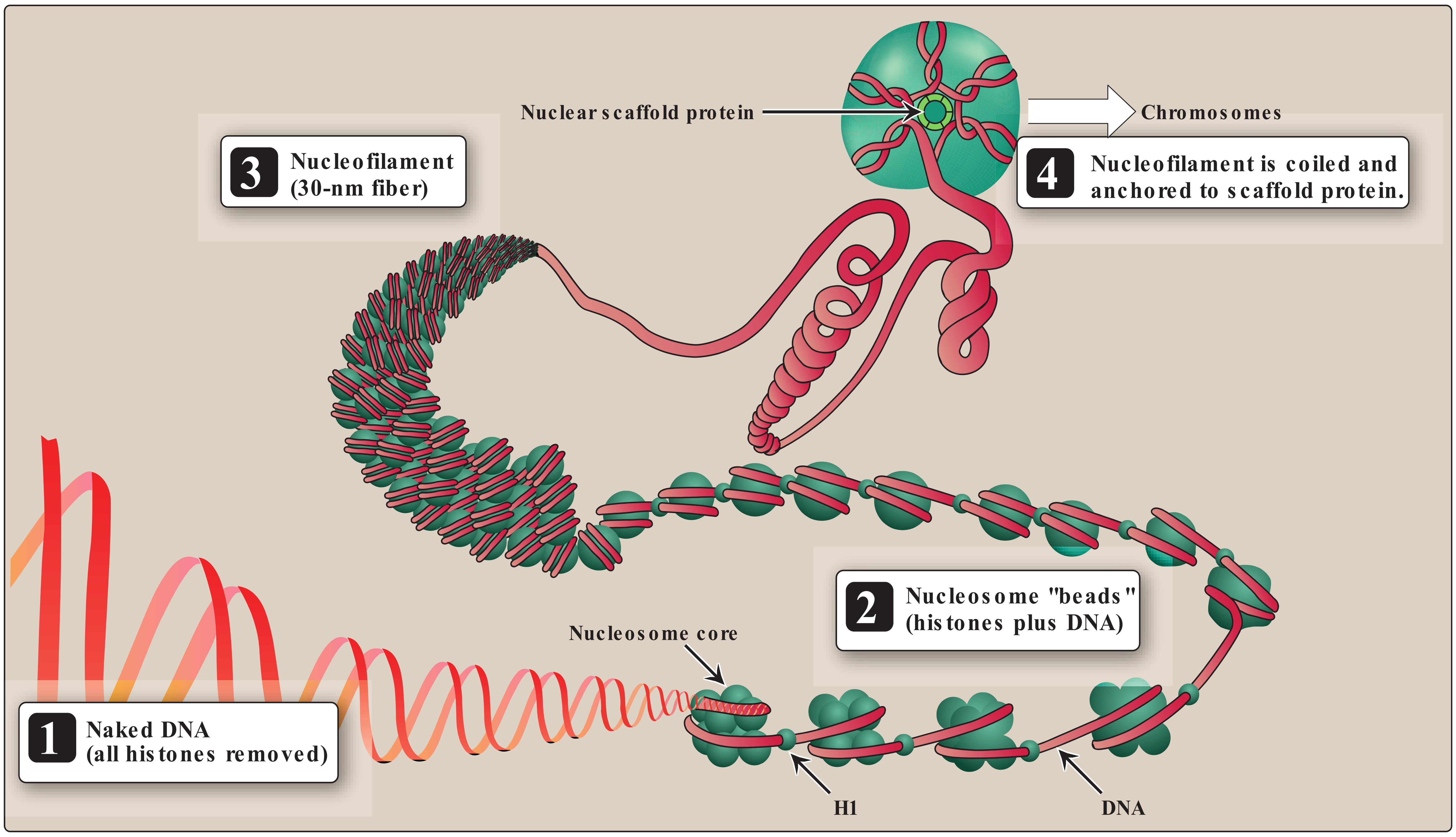

Nuclear scaffold protein
Chromosomes
3 Nucleofilament (30-nm fiber)
4 Nucleofilament is coiled and anchored to scaffold protein.
2 Nucleosome "beads" (histones plus DNA)
Nucleosome core
1 Naked DNA (all histones removed)
H1
DNA

Figure 29.27
Structural organization of eukaryotic DNA. [Note: A 10^4 compaction is seen from 1 to 4.] H = histone.

2. **Higher levels of organization:** Nucleosomes can be packed more tightly to form a polynucleosome (also called a nucleofilament). This structure assumes the shape of a coil, often referred to as a 30-nm fiber. The fiber is organized into loops that are anchored by a nuclear scaffold containing several proteins. Additional levels of organization lead to the final chromosomal structure (Figure 29.27).

B. Fate of nucleosomes during DNA replication

Parental nucleosomes are disassembled to allow access to DNA during replication. Once DNA is synthesized, nucleosomes form rapidly. Their histone proteins come both from new synthesis and from the transfer of intact parental histone octamers.

VI. DNA REPAIR

Despite the elaborate proofreading system employed during DNA synthesis, errors (including incorrect base-pairing or insertion of one to a few extra nucleotides) can occur. In addition, DNA is constantly being subjected to environmental insults that cause the alteration or removal of nucleotide bases. The damaging agents can be either chemicals (for example, nitrous acid, which can deaminate bases), or radiation (for example, non-ionizing ultraviolet light, which can fuse two pyrimidines adjacent to each other in the DNA, and high-energy ionizing radiation, which can cause double-strand breaks). Bases are also altered or lost spontaneously from mammalian DNA at a rate of many thousands per cell per day. If the damage is not repaired, a permanent change (mutation) is introduced that can result in any of a number of deleterious effects, including loss of control over the proliferation of the mutated cell, leading to cancer. Luckily, cells are remarkably efficient at repairing damage done to their DNA. Most of the repair systems involve recognition of the damage (lesion) on the DNA, removal or excision of the damage, replacement or filling the gap left by excision using the sister strand as a template for DNA synthesis, and ligation. These excision repair systems remove one to tens of nucleotides. [Note: Repair synthesis of DNA can occur outside of the S phase.]

A. Repair of mismatched bases (mismatch repair)

Sometimes replication errors escape the proofreading function during DNA synthesis, causing a mismatch of one to several bases. In E. coli, mismatch repair (MMR) is mediated by a group of proteins known as the Mut proteins (Figure 29.28). Homologous proteins are present in humans. [Note: MMR reduces the error rate of replication from one in ten million to one in a billion.]

1. **Identification of the mismatched strand:** When a mismatch occurs, the Mut proteins that identify the mispaired nucleotide(s) must be able to discriminate between the correct strand and the strand with the mismatch. Discrimination is based on the degree of methylation. GATC sequences, which are found approximately once every thousand nucleotides, are methylated on the adenine (A) residue. This methylation is not done immediately after synthesis, so the newly synthesized DNA is hemimethylated (that is, the parental strand is methylated, but the daughter strand is not). The methylated parental strand is assumed to be correct, and it is the daughter strand that gets repaired. [Note: The exact mechanism by which the daughter strand is identified in eukaryotes is not yet known.]

2. **Repair of damaged DNA:** When the strand containing the mismatch is identified, an endonuclease nicks the strand, and the mismatched nucleotide(s) is/are removed by an exonuclease. Additional nucleotides at the 5'- and 3'-ends of the mismatch are also removed. The gap left by removal of the nucleotides is filled, using the sister strand as a template, by a DNA polymerase, typically DNA pol I. The 3'-hydroxyl of the newly synthesized DNA is joined to the 5'-phosphate of the remaining stretch of the original DNA strand by DNA ligase (see p. 406).

Mutation to the proteins involved in mismatch repair in humans is associated with hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome. With HNPCC, there is an increased risk for developing colon cancer (as well as other cancers); however, only about 5% of all colon cancer is the result of mutations in mismatch repair.

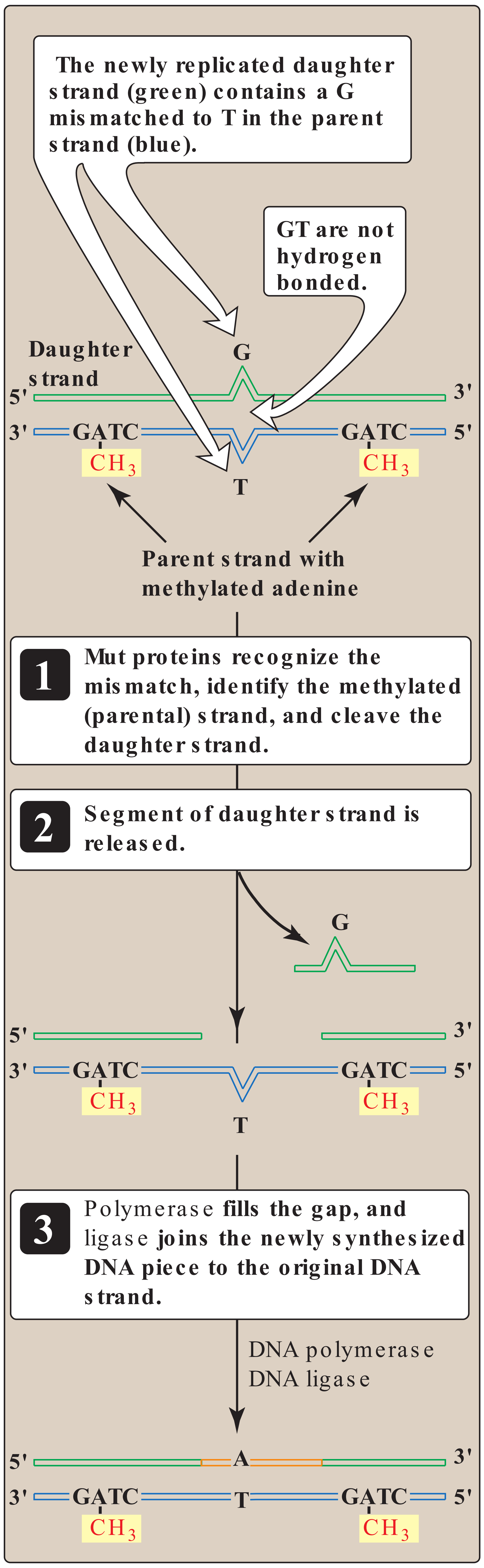

The newly replicated daughter strand (green) contains a G mismatched to T in the parent strand (blue).
GT are not hydrogen bonded.
Daughter strand
G
5'
3'
3'
GATC
GATC
5'
CH3
CH3
T
Parent strand with methylated adenine
1 Mut proteins recognize the mismatch, identify the methylated (parental) strand, and cleave the daughter strand.
2 Segment of daughter strand is released.
G
5'
3'
3'
GATC
GATC
5'
CH3
CH3
T
3 Polymerase fills the gap, and ligase joins the newly synthesized DNA piece to the original DNA strand.
DNA polymerase
DNA ligase
5'
A
3'
3'
GATC
T
GATC
5'
CH3
CH3

Figure 29.28
Methyl-directed mismatch repair in E. coli. [Note: The Mut proteins, S and L, recognize the mismatch and identify the parental (methylated) strand, and Mut H cleaves the daughter strand.] A = adenine; C= cytosine; G = guanine; T = thymine.

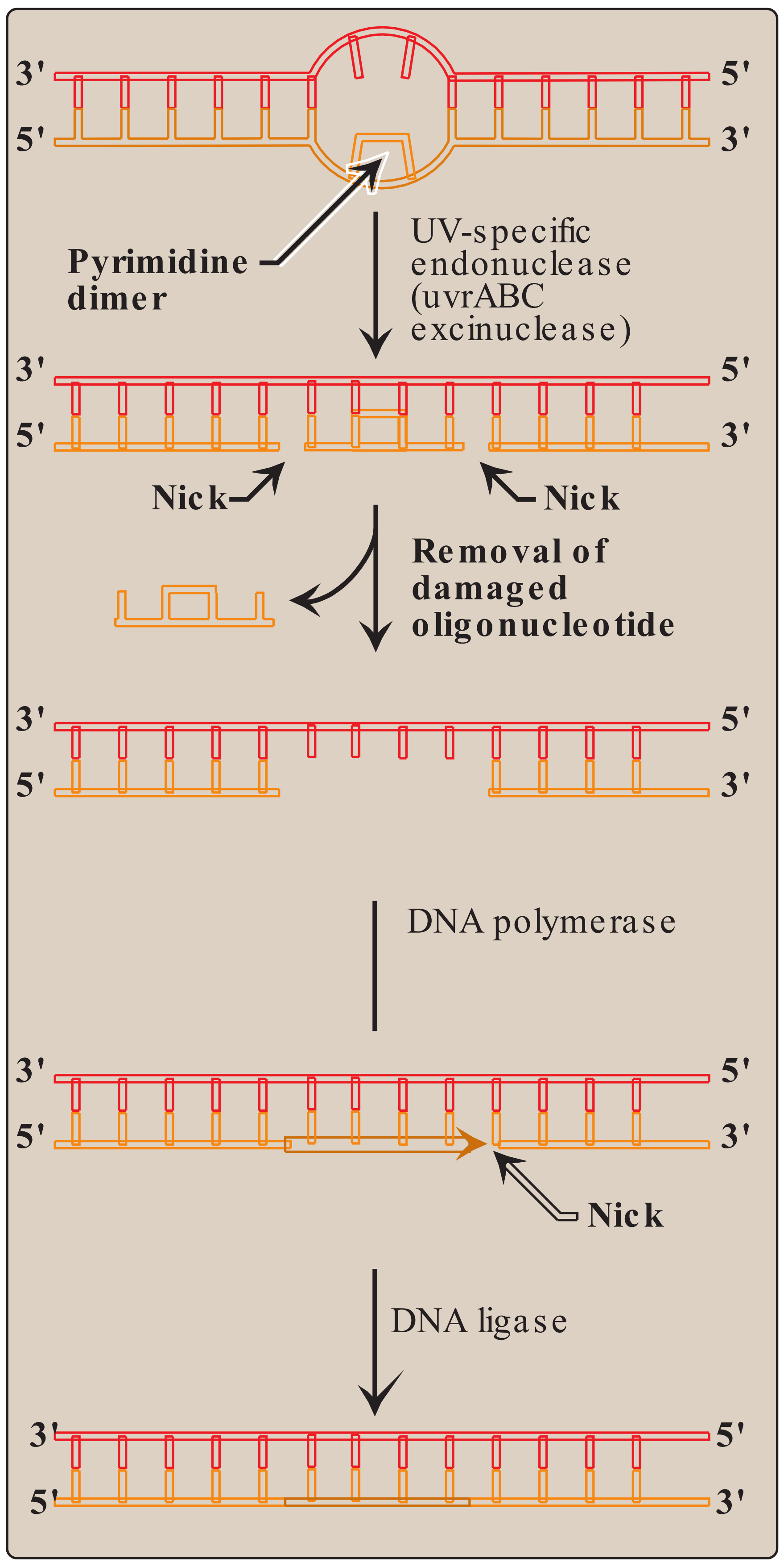


Figure 29.29
Nucleotide excision repair of pyrimidine dimers in E. coli DNA. UV = ultraviolet.

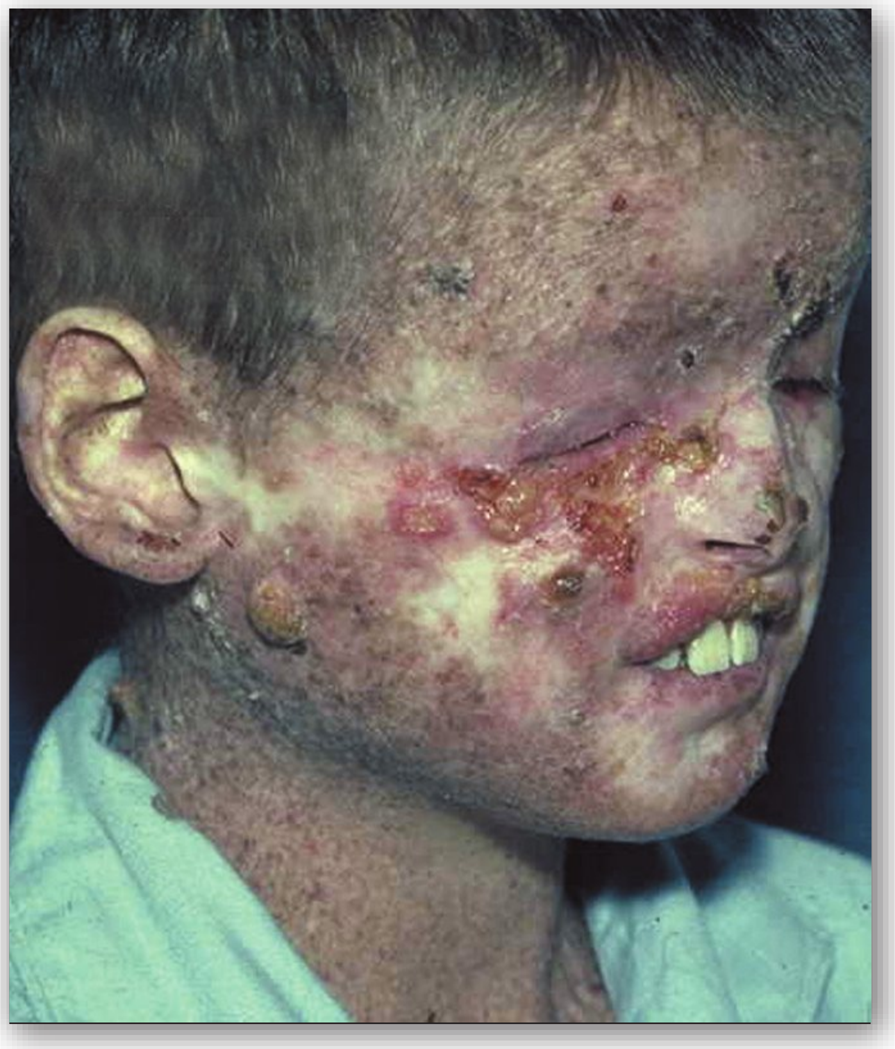


Figure 29.30
Patient with xeroderma pigmentosum.

B. Repair of damage caused by ultraviolet light (nucleotide excision repair)

Exposure of a cell to ultrtaviolet (UV) light can result in the covalent joining of two adjacent pyrimidines (usually thymines), producing a dimer. These thymine dimers prevent DNA pol from replicating the DNA strand beyond the site of dimer formation. Thymine dimers are excised in bacteria by UvrABC proteins in a process known as nucleotide excision repair (NER) as illustrated in Figure 29.29. A related pathway is present in humans (see below).

1. **Recognition and excision of dimers by UV-specific endonuclease:** First, a UV-specific endonuclease (called uvrABC excinuclease) recognizes the dimer and cleaves the damaged strand on both the 5'-side and 3'-side of the dimer. A short oligonucleotide containing the dimer is released, leaving a gap in the DNA strand that formerly contained the dimer. This gap is filled in using a DNA polymerase and DNA ligase.

2. **UV radiation and cancer:** Pyrimidine dimers can be formed in the skin cells of humans exposed to unfiltered sunlight. In the rare genetic disease xeroderma pigmentosum (XP), the cells cannot repair the damaged DNA, resulting in extensive accumulation of mutations and, consequently, early and numerous skin cancers (Figure 29.30). XP can be caused by defects in any of the several genes that code for the XP proteins required for nucleotide excision repair of UV damage in humans.

C. Repair of base alterations (base excision repair)

The bases of DNA can be altered, either spontaneously, as is the case with cytosine, which slowly undergoes deamination (the loss of its amino group) to form uracil, or by the action of deaminating or alkylating compounds. For example, nitrous acid, which is formed by the cell from precursors such as the nitrosamines, nitrites, and nitrates, is a potent compound that deaminates cytosine, adenine (to hypoxanthine), and guanine (to xanthine). Bases can also be lost spontaneously. For example, approximately 10,000 purine bases are lost this way per cell per day. Lesions involving base alterations or loss can be corrected by base excision repair ([BER] Figure 29.31).

1. **Removal of abnormal bases:** In BER, abnormal bases, such as uracil, which can occur in DNA either by deamination of cytosine or improper use of dUTP instead of dTTP during DNA synthesis, are recognized by specific glycosylases that hydrolytically cleave them from the deoxyribose–phosphate backbone of the strand. This leaves an apyrimidinic site (or apurinic, if a purine was removed), both referred to as AP sites.

2. **Recognition and repair of an AP site:** Specific AP-endonucleases recognize that a base is missing and initiate the process of excision and gap-filling by making an endonucleolytic cut just to the 5'-side of the AP site. A deoxyribose phosphate lyase removes the single, base-free, sugar phosphate residue. DNA polymerase and DNA ligase complete the repair process.

D. Repair of double-strand breaks

Ionizing radiation or oxidative free radicals (see p. 148) can cause double-strand breaks in DNA that are potentially lethal to the cell. Such breaks also occur naturally during gene rearrangements. dsDNA breaks cannot be corrected by the previously described strategy of excising the damage on one strand and using the remaining strand as a template for replacing the missing nucleotide(s). Instead, they are repaired by one of two systems. The first is nonhomologous end-joining (NHEJ), in which a group of proteins mediates the recognition, processing, and ligation of the ends of two DNA fragments. However, some DNA is lost in the process. Consequently, this mechanism of repair is error prone and mutagenic. Defects in this repair system are associated with a predisposition to cancer and immunodeficiency syndromes. The second repair system, homologous recombination (HR), uses the enzymes that normally perform genetic recombination between homologous chromosomes during meiosis. This system is much less error prone than NHEJ because any DNA that was lost is replaced using homologous DNA as a template. [Note: Mutations to the proteins, BRCA1 or 2 (breast cancer 1 or 2), which are involved in HR, increase the risk for developing breast cancer.]

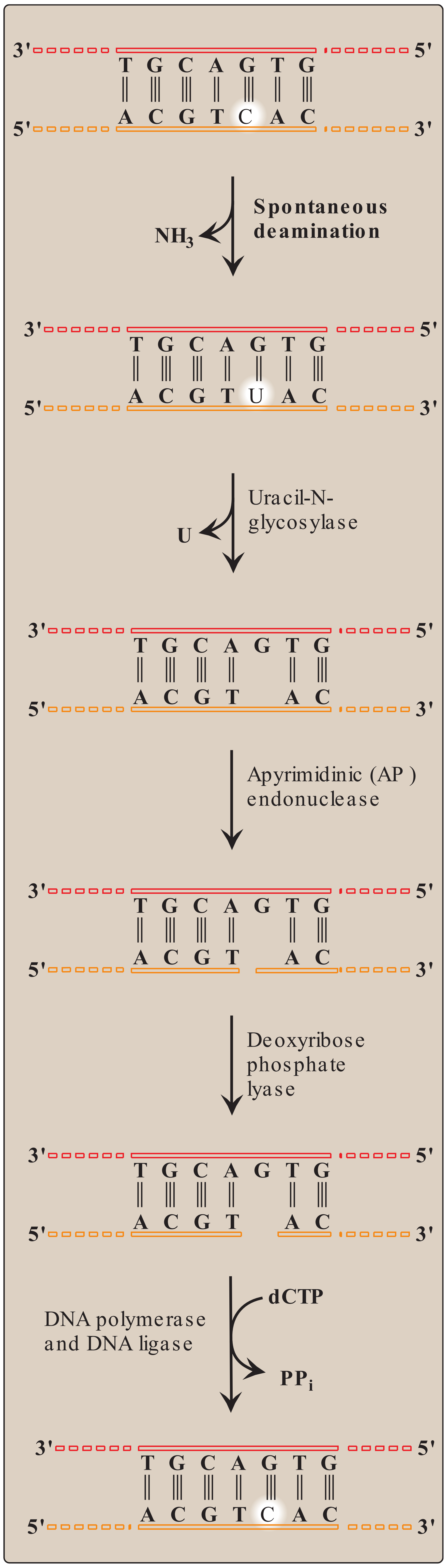


Figure 29.31
Correction of base alterations by base excision repair. T= thymine; A = adenine; C = cytosine; G = guanine; PP_i = pyrophosphate.

VII. CHAPTER SUMMARY

DNA is a polymer of deoxyribonucleoside monophosphates covalently linked by **3'→5'-phosphodiester bonds** (Figure 29.32). The resulting long, unbranched chain has **polarity**, with both a 5'-end and a 3'-end. The sequence of nucleotides is read 5'→3'. DNA exists as a **double-stranded** molecule, in which the two chains are paired in an **antiparallel** manner, and wind around each other, forming a **double helix**. **Adenine** pairs with **thymine**, and **cytosine** pairs with **guanine**. Each strand of the double helix serves as a **template** for constructing a **complementary** daughter strand (**semiconservative replication**). DNA replication occurs in the **S phase** of the cell cycle and begins at the **origin of replication**. As the two strands unwind and separate, synthesis occurs at two replication forks that move away from the origin in opposite directions (bidirectionally). Helicase unwinds the double helix. As the two strands of the double helix are separated, **positive supercoils** are produced in the region of DNA ahead of the replication fork and negative supercoils behind the fork. **DNA** topoisomerases **types I and II** remove supercoils. DNA polymerases **(**pol**)** synthesize **n**ew DNA strands only in the **5'→3'** direction. Therefore, one of the newly synthesized stretches of nucleotide chains must grow in the 5'→3' direction toward the replication fork (**leading strand**) and one in the 5'→3' direction away from the replication fork (**lagging strand**). DNA pols require a **primer**. The primer for de novo DNA synthesis is a short stretch of RNA synthesized by primase. The leading strand only needs one RNA primer, whereas the lagging strand needs many. In E. coli, DNA chain elongation is catalyzed by DNA pol III, using **5'-deoxyribonucleoside triphosphates** as substrates. The enzyme **"proofreads"** the newly

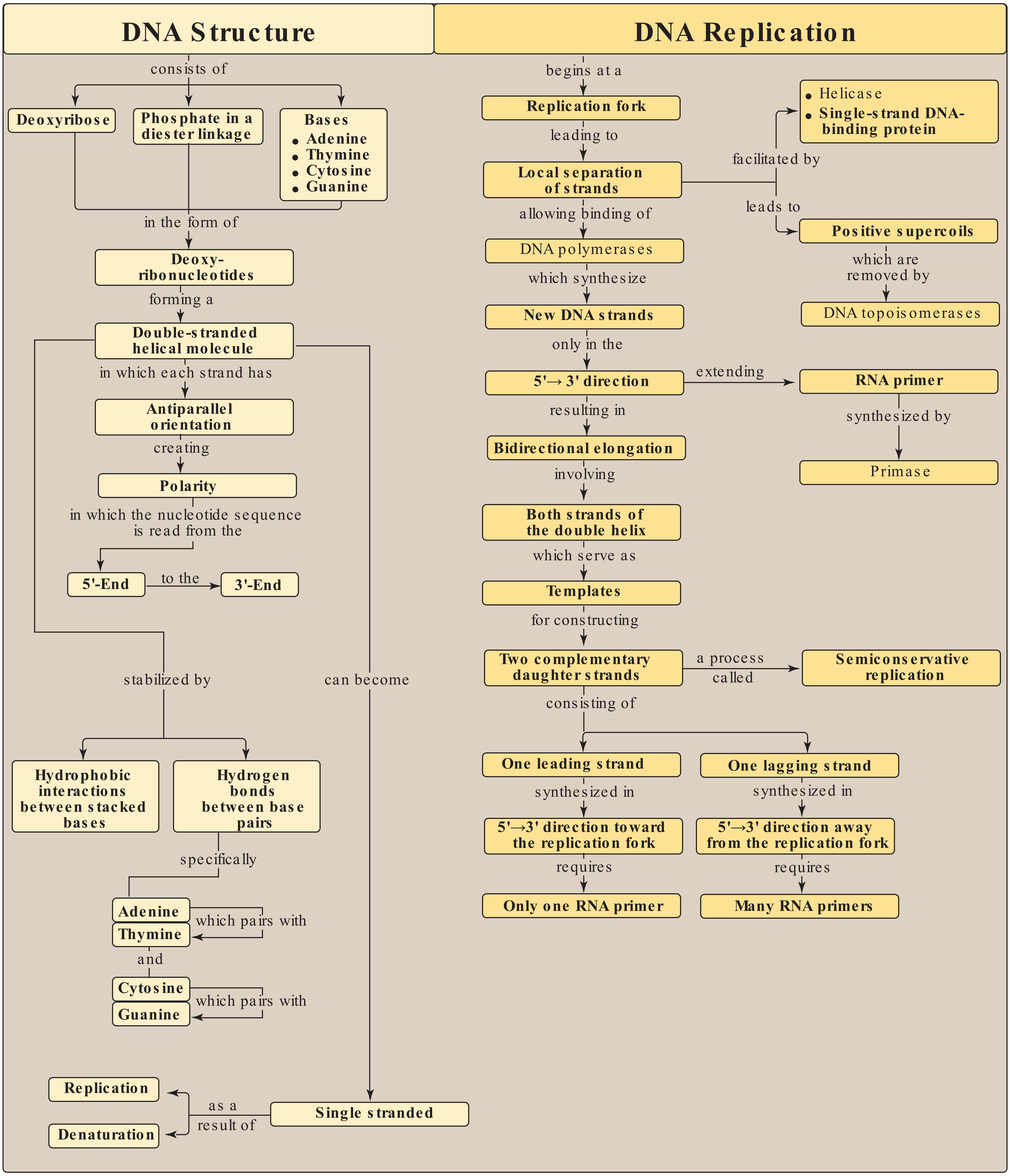

DNA Structure
consists of
Deoxyribose
Phosphate in a diester linkage
Bases
• Adenine
• Thymine
• Cytosine
• Guanine
in the form of
Deoxy-ribonucleotides
forming a
Double-stranded helical molecule
in which each strand has
Antiparallel orientation
creating
Polarity
in which the nucleotide sequence is read from the
5'-End
to the
3'-End
stabilized by
Hydrophobic interactions between stacked bases
Hydrogen bonds between base pairs
specifically
Adenine
which pairs with
Thymine
and
Cytosine
which pairs with
Guanine
can become
Single stranded
as a result of
Replication
Denaturation
DNA Replication
begins at a
Replication fork
leading to
Local separation of strands
facilitated by
• Helicase
• Single-strand DNA-binding protein
leads to
Positive supercoils
which are removed by
DNA topoisomerases
allowing binding of
DNA polymerases
which synthesize
New DNA strands
only in the
5'→ 3' direction
extending
RNA primer
synthesized by
Primase
resulting in
Bidirectional elongation
involving
Both strands of the double helix
which serve as
Templates
for constructing
Two complementary daughter strands
a process called
Semiconservative replication
consisting of
One leading strand
synthesized in
5'→3' direction toward the replication fork
requires
Only one RNA primer
One lagging strand
synthesized in
5'→3' direction away from the replication fork
requires
Many RNA primers

Figure 29.32 (continued on next page)
Key concept map for DNA structure, replication, and repair.

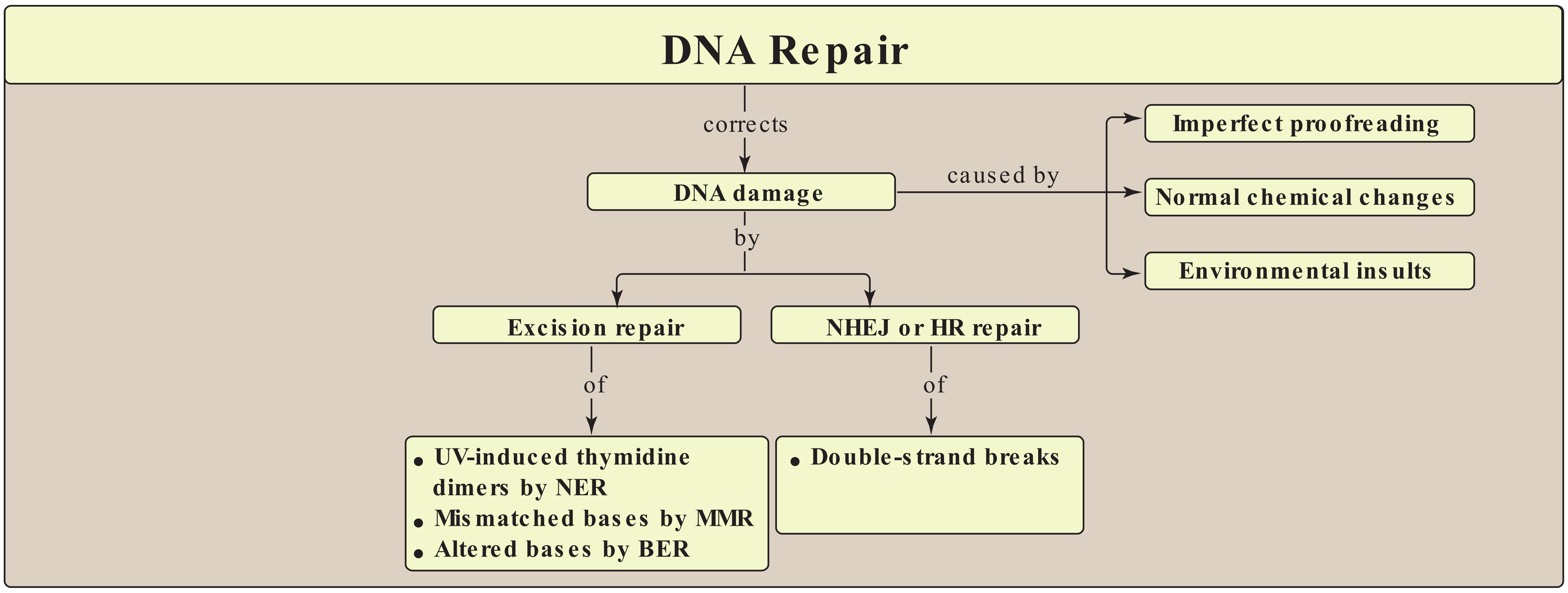


Figure 29.32 (continued from previous page)
Key concept map for DNA structure, replication, and repair. NHEJ = nonhomologous end-joining; HR = homologous recombination; NER = nucleotide excision repair; MMR = mismatch repair; BER = base excision repair; UV = ultraviolet light.

synthesized DNA, removing terminal mismatched nucleotides with its 3'→5' exonuclease activity. RNA primers are removed by DNA pol I, using its 5'→3' exonuclease activity. This enzyme fills the gaps with DNA, proofreading as it synthesizes. The final phosphodiester linkage is catalyzed by DNA ligase. There are at least five high-fidelity **eukaryotic** DNA polymerases. Pol α is a multisubunit enzyme, one subunit of which is a primase. Pol *α* 5'→3' polymerase activity adds a short piece of DNA to the RNA primer. Pol □completes DNA synthesis on the leading strand, whereas pol □elongates each lagging strand fragment. Pol β is involved with DNA repair, and pol γ replicates mitochondrial DNA. Pols *ε*, *d*, and *γ* use 3'→5' exonuclease activity to proofread. **Nucleoside analogs** containing modified sugars can be used to block DNA chain growth. They are useful in anticancer and antiviral chemotherapy. **Telomeres** are stretches of **highly repetitive DNA** complexed with protein that protect the **ends** of linear chromosomes. As most cells divide and age, these sequences are shortened, contributing to senescence. In cells that do not senesce (for example, germline and cancer cells), **telomerase** employs its enzyme component reverse transcriptase to extend the telomeres, using its **RNA** as a **template**. There are five classes of **positively charged histone (H) proteins**. Two each of histones H2A, H2B, H3, and H4 form an octameric structural core around which DNA is wrapped creating a **nucleosome**. The DNA connecting the nucleosomes, called **linker DNA**, is bound to H1. Nucleosomes can be packed more tightly to form a nucleofilament. Additional levels of organization create a chromosome. Most DNA damage can be corrected by **excision repair** involving recognition and removal of the damage by repair proteins, followed by replacement in E. coli by DNA pol and joining by ligase. **Ultraviolet light** can cause thymine dimers that are recognized and removed by **uvrABC proteins of nucleotide excision repair.** Defects in the **XP proteins** needed for thymine dimer repair in humans result in **xeroderma pigmentosum**. **Mismatched** bases are repaired by a similar process of recognition and removal by **Mut proteins** in E. coli. The extent of **methylation** is used for strand identification in prokaryotes. Defective mismatch repair by homologous proteins in humans is associated with **hereditary nonpolyposis colorectal cancer**. Abnormal bases (such as uracil) are removed by glycosylases in **base excision repair**, and the sugar phosphate at the apyrimidinic or apurinic (AP) site is cut out. Double-strand breaks in DNA are repaired by **nonhomologous end-joining** (error prone) and **homologous recombination**.

RNA Structure, Synthesis, and Processing

30

I. OVERVIEW

The genetic master plan of an organism is contained in the sequence of deoxyribonucleotides in its deoxyribonucleic acid (DNA). However, it is through the ribonucleic acid (RNA), the "working copies" of the DNA, that the master plan is expressed (Figure 30.1). The copying process, during which a DNA strand serves as a template for the synthesis of RNA, is called transcription. Transcription produces messenger RNAs (mRNAs) that are translated into sequences of amino acids (polypeptide chains or proteins) and ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), and additional small RNA molecules that perform specialized structural, catalytic, and regulatory functions and are not translated. That is, they are noncoding RNAs (ncRNAs). [Note: Only about 2% of the genome codes for proteins.] The final product of gene expression, therefore, can be RNA or protein, depending upon the gene. A central feature of transcription is that it is highly selective. For example, many transcripts are made of some regions of the DNA. In other regions, few or no transcripts are made. This selectivity is due, at least in part, to signals embedded in the nucleotide sequence of the DNA. These signals instruct the RNA polymerase where to start, how often to start, and where to stop transcription. A variety of regulatory proteins is also involved in this selection process. The biochemical differentiation of an organism's tissues is ultimately a result of the selectivity of the transcription process. [Note: This selectivity of transcription is in contrast to the "all-or-none" nature of genomic replication.] Another important feature of transcription is that many RNA transcripts that initially are faithful copies of one of the two DNA strands may undergo various modifications, such as terminal additions, base modifications, trimming, and internal segment removal, which convert the inactive primary transcript into a functional molecule.

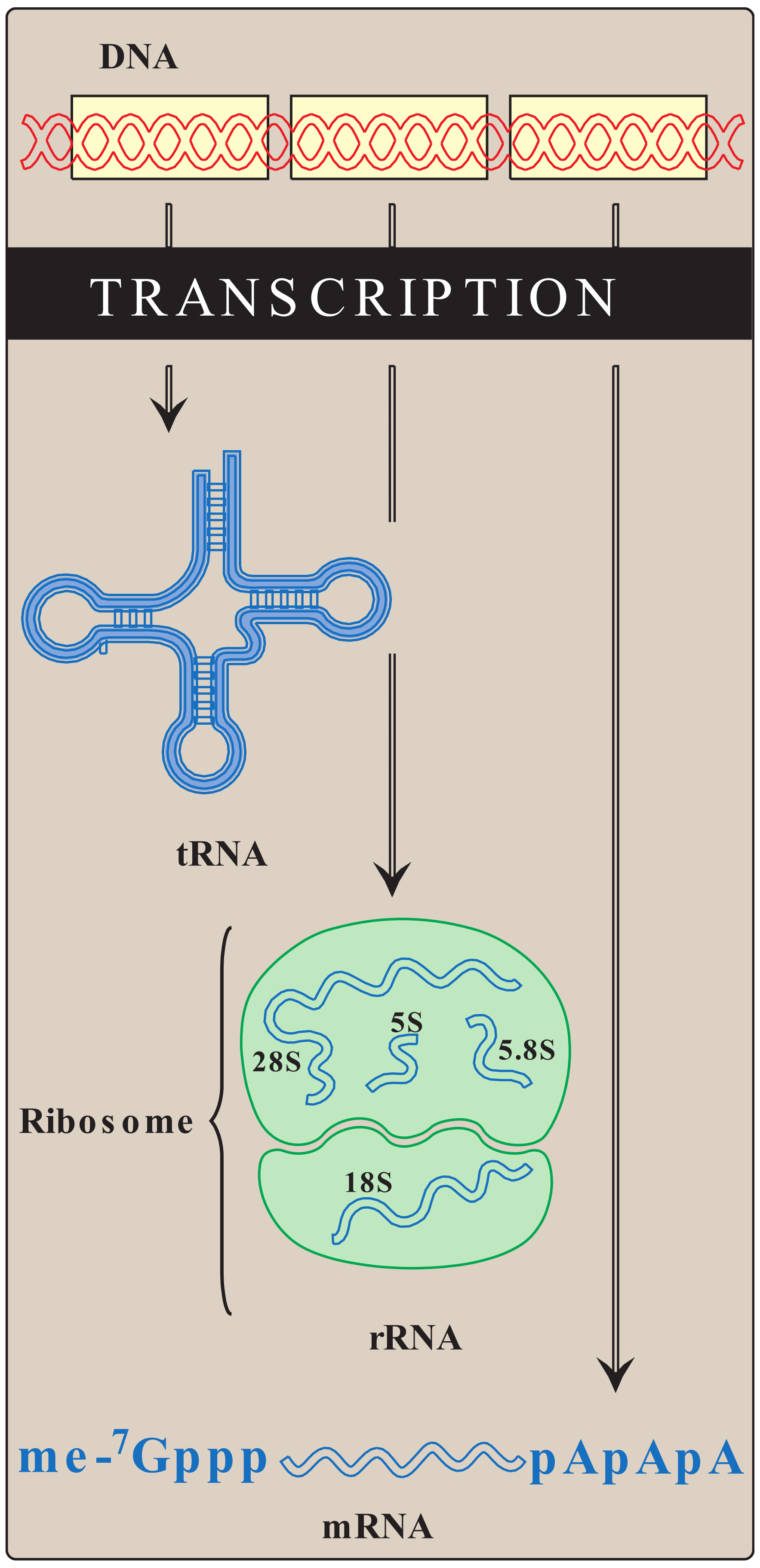


Figure 30.1
Expression of genetic information by transcription. [Note: RNAs shown are eukaryotic.] tRNA = transfer RNA; rRNA = ribosomal RNA; mRNA = messenger RNA; me-^{7}Gppp = 7-methylguanosine triphosphate "cap;" AAA = poly-A "tail," each described on p. 418.

II. STRUCTURE OF RNA

There are three major types of RNA that participate in the process of protein synthesis: rRNA, tRNA, and mRNA. Like DNA, these three types of RNA are unbranched polymeric molecules composed of nucleoside monophosphates joined together by 3'→5'-phosphodiester bonds (see

p. 396). However, they differ from DNA in several ways. For example, they are considerably smaller than DNA, contain ribose instead of deoxyribose and uracil instead of thymine, and exist as single strands that are capable of folding into complex structures. The three major types of RNA also differ from each other in size, function, and special structural modifications. [Note: In eukaryotes, additional small ncRNA molecules found in the nucleolus (snoRNAs), nucleus (snRNA), and cytoplasm (miRNA) perform specialized functions as described on pp. 425 , 426, and 459.]

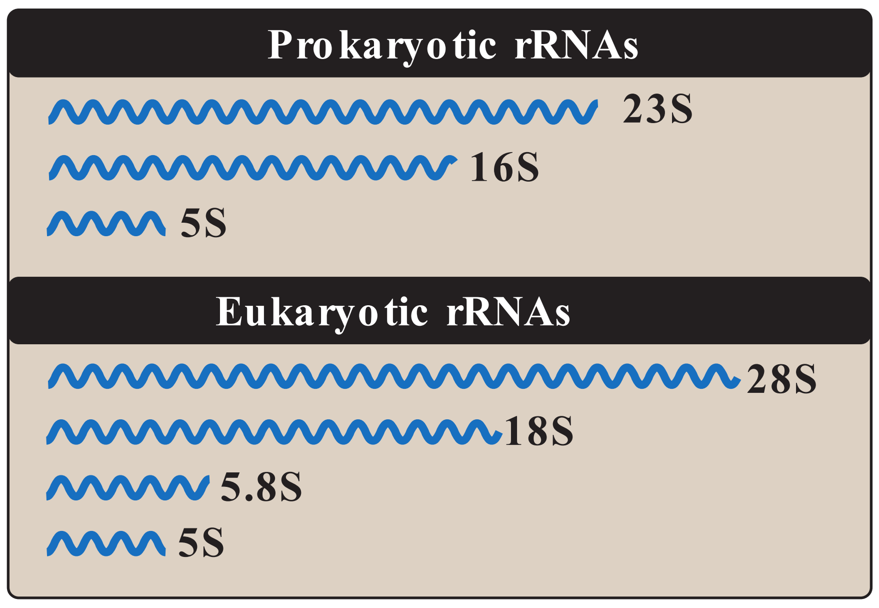


Figure 30.2
Prokaryotic and eukaryotic ribosomal RNAs (rRNAs). S = Svedberg unit.

A. Ribosomal RNA

rRNAs are found in association with several proteins as components of the ribosomes, the complex structures that serve as the sites for protein synthesis (see p. 436). There are three distinct size species of rRNA (23S, 16S, and 5S) in prokaryotic cells (Figure 30.2). In the eukaryotic cytosol, there are four rRNA species (28S, 18S, 5.8S, and 5S, where "S" is the Svedberg unit for sedimentation rate, which is determined by the size and shape of the particle.) Together, rRNAs make up about 80% of the total RNA in the cell. [Note: Some RNAs function as catalysts, for example, an rRNA in protein synthesis (see p. 439). RNA with catalytic activity is termed a "ribozyme."]

B. Transfer RNA

tRNAs are the smallest (4S) of the three major types of RNA molecules. There is at least one specific type of tRNA molecule for each of the 20 amino acids commonly found in proteins. Together, tRNAs make up about 15% of the total RNA in the cell. The tRNA molecules contain a high percentage of unusual bases, for example, dihydrouracil (see Figure 22.2, p. 292) and have extensive intrachain base-pairing (Figure 30.3) that leads to characteristic secondary and tertiary structure. Each tRNA serves as an "adaptor" molecule that carries its specific amino acid, covalently attached to its 3'-end, to the site of protein synthesis. There it recognizes the genetic code sequence on an mRNA, which specifies the addition of its amino acid to the growing peptide chain (see p. 432).

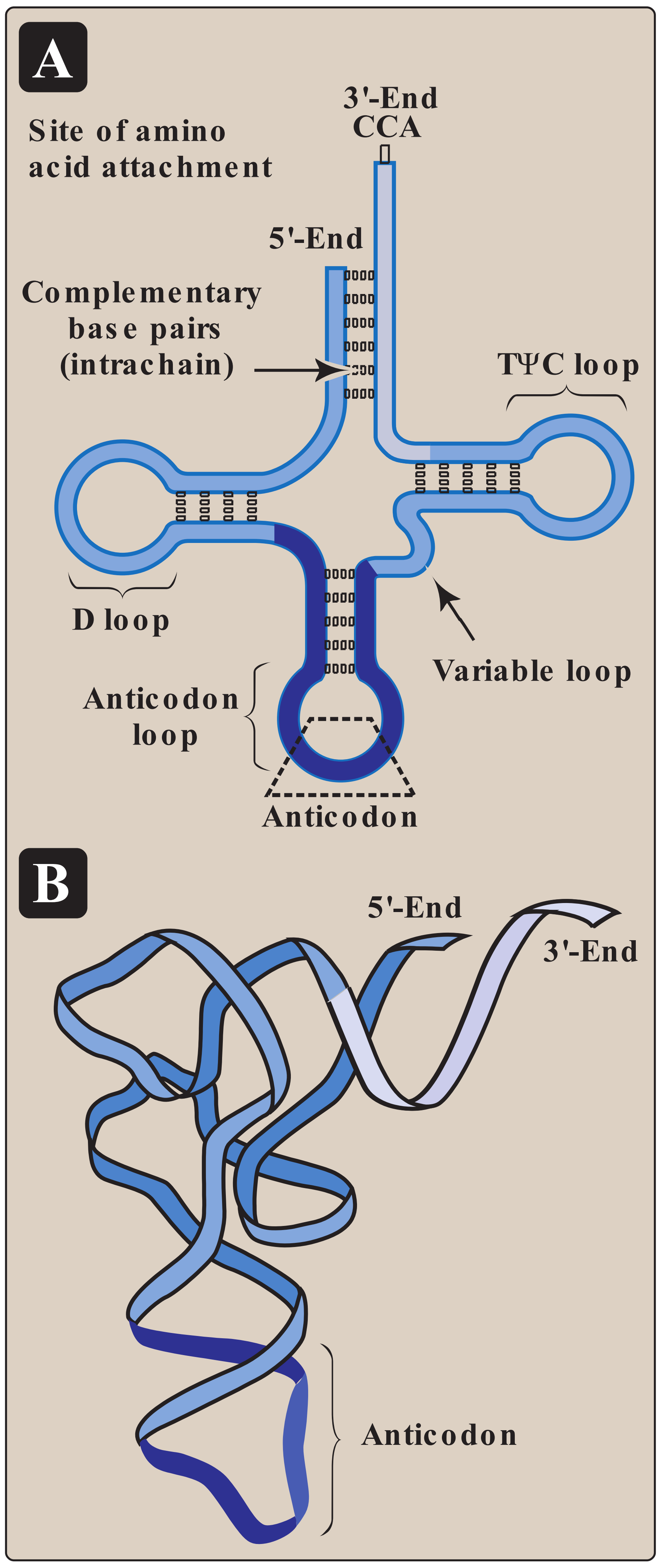


Figure 30.3
A. Characteristic transfer RNA (tRNA) secondary structure (cloverleaf).
B. Folded (tertiary) tRNA structure found in cells. D = dihydrouracil; Ψ = pseudouracil; T = thymine; C = cytosine; A = adenine.

C. Messenger RNA

mRNA comprises only about 5% of the RNA in the cell, yet is by far the most heterogeneous type of RNA in size and base sequence. mRNA carries genetic information from DNA for use in protein synthesis. In eukaryotes, this involves transfer of mRNA out of the nucleus and into the cytosol. If the mRNA carries information from more than one gene, it is said to be polycistronic (cistron = gene). Polycistronic mRNA is characteristic of prokaryotes. If the mRNA carries information from just one gene, it is said to be monocistronic and is characteristic of eukaryotes. In addition to the protein-coding regions that can be translated, mRNA contains untranslated regions at its 5'- and 3'-ends (Figure 30.4). Special structural characteristics of eukaryotic (but not prokaryotic) mRNA include a long sequence of adenine nucleotides (a poly-A "tail") on the 3'-end of the RNA chain, plus a "cap" on the 5'-end consisting of a molecule of 7-methylguanosine attached through an unusual (5'→5') triphosphate linkage. The mechanisms for modifying mRNA to create these special structural characteristics are discussed on p. 425.

III. TRANSCRIPTION OF PROKARYOTIC GENES

The structure of RNA polymerase (RNA pol), the signals that control transcription, and the varieties of modification that RNA transcripts can undergo differ among organisms, and particularly from prokaryotes to eukaryotes. Therefore, the discussions of prokaryotic and eukaryotic transcription are presented separately.

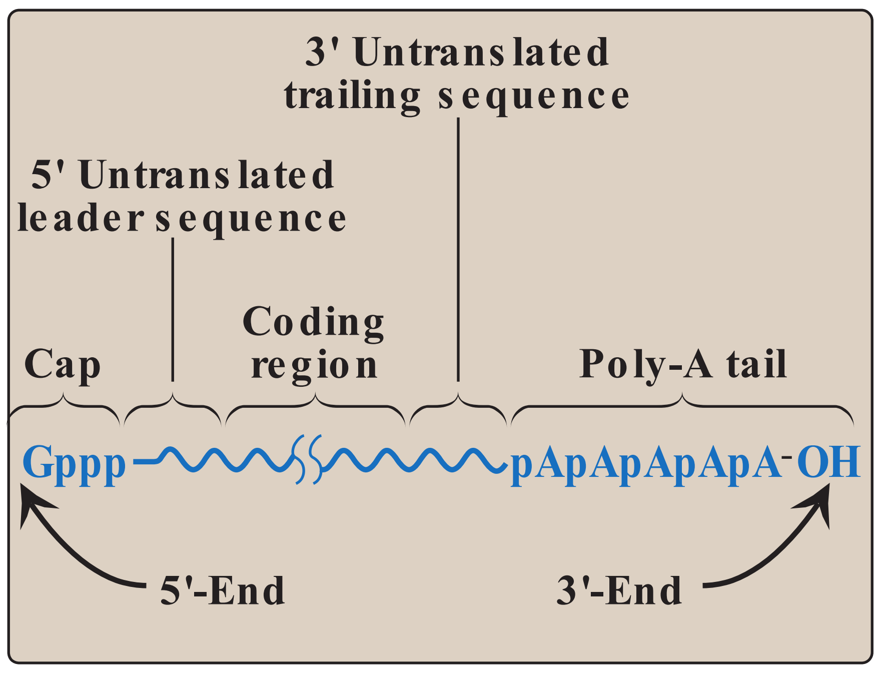

3' Untranslated trailing sequence
5' Untranslated leader sequence
Cap
Coding region
Poly-A tail
Gppp
pApApApApA-OH
5'-End
3'-End

Figure 30.4
Structure of eukaryotic messenger RNA.

A. Properties of prokaryotic RNA polymerase

In bacteria, one species of RNA pol synthesizes all of the RNA except for the short RNA primers needed for DNA replication [Note: RNA primers are synthesized by a specialized enzyme, primase (see p. 402).] RNA pol is a multisubunit enzyme that recognizes a nucleotide sequence (the promoter region) at the beginning of a length of DNA that is to be transcribed. It next makes a complementary RNA copy of the DNA template strand, and then recognizes the end of the DNA sequence to be transcribed (the termination region). RNA is synthesized from its 5'-end to its 3'-end, antiparallel to its DNA template strand (see p. 397). The template is copied as it is in DNA synthesis, in which a guanine (G) on the DNA specifies a cytosine (C) in the RNA, a C specifies a G, a thymine (T) specifies an adenine (A), but an A specifies a uracil (U) instead of a T (Figure 30.5). The RNA, then, is complementary to the DNA template (antisense, minus) strand and identical to the coding (sense, plus) strand, with U replacing T. Within the DNA molecule, regions of both strands can serve as templates for transcription. For a given gene, however, only one of the two DNA strands can be the template. Which strand is used is determined by the location of the promoter for that gene. Transcription by RNA pol involves a core enzyme and several auxiliary proteins:

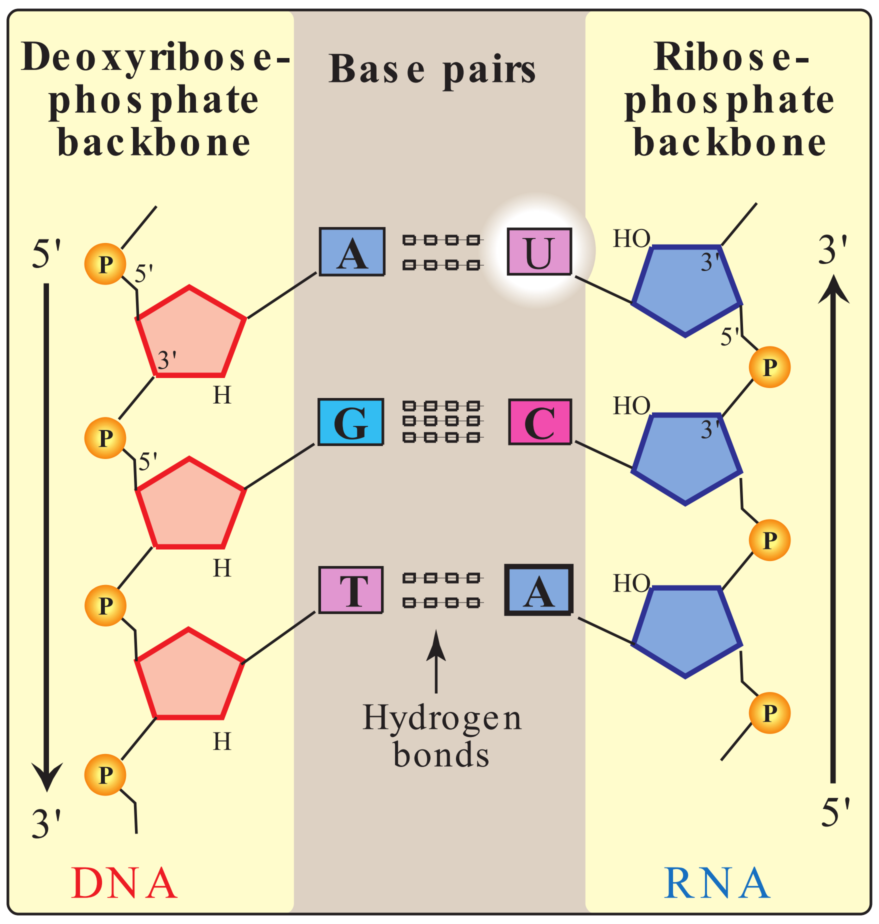


Figure 30.5
Antiparallel, complementary base pairs between DNA and RNA.
T= thymine; A = adenine;
C = cytosine; G = guanine; U = uracil.

1. **Core enzyme:** Five of the enzyme's peptide subunits, 2α, 1b, 1b', and 1W, are required for enzyme assembly (α, W) template binding (b'), and the 5'→3' RNA polymerase activity (b), and are referred to as the core enzyme (Figure 30.6). However, this enzyme lacks specificity (that is, it cannot recognize the promoter region on the DNA template).

2. **Holoenzyme:** The s subunit ("sigma factor") enables RNA pol to recognize promoter regions on the DNA. The s subunit plus the core enzyme make up the holoenzyme. [Note: Different s factors recognize different groups of genes.]

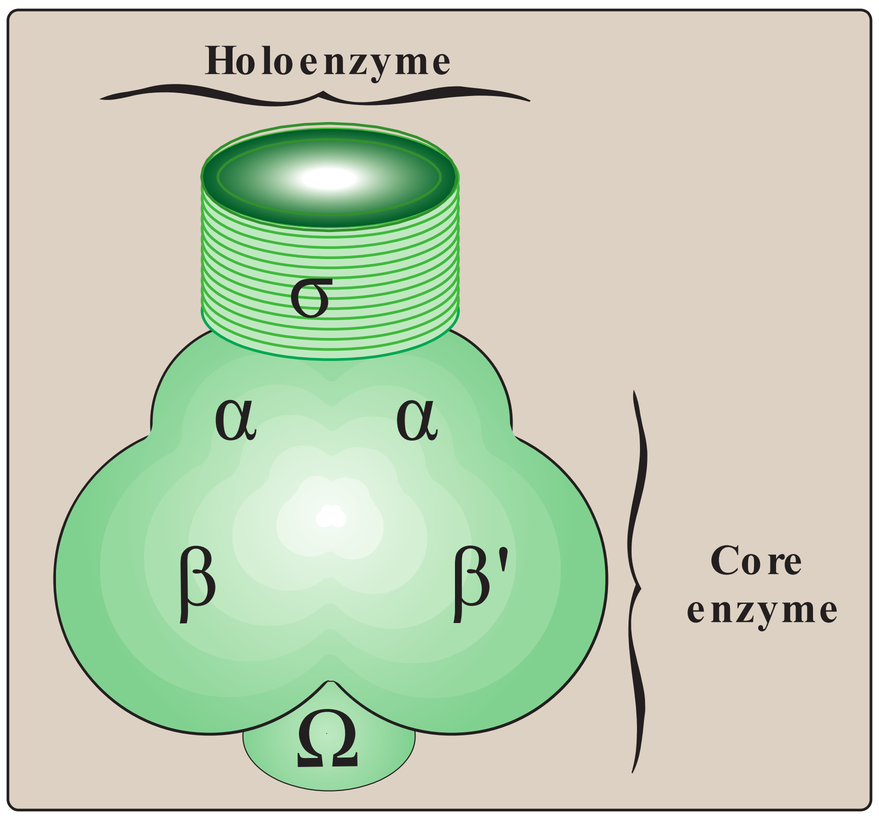


Figure 30.6
Components of prokaryotic RNA polymerase.

B. Steps in RNA synthesis

The process of transcription of a typical gene of Escherichia coli (E. coli) can be divided into three phases: initiation, elongation, and termination. A transcription unit extends from the promoter to the termination region, and the initial product of transcription by RNA pol is termed the primary transcript.

1. **Initiation:** Transcription begins with the binding of the RNA pol holoenzyme to a region of the DNA known as the promoter, which is not transcribed. The prokaryotic promoter contains characteristic consensus sequences (Figure 30.7). [Note: Consensus sequences are idealized sequences in which the base shown

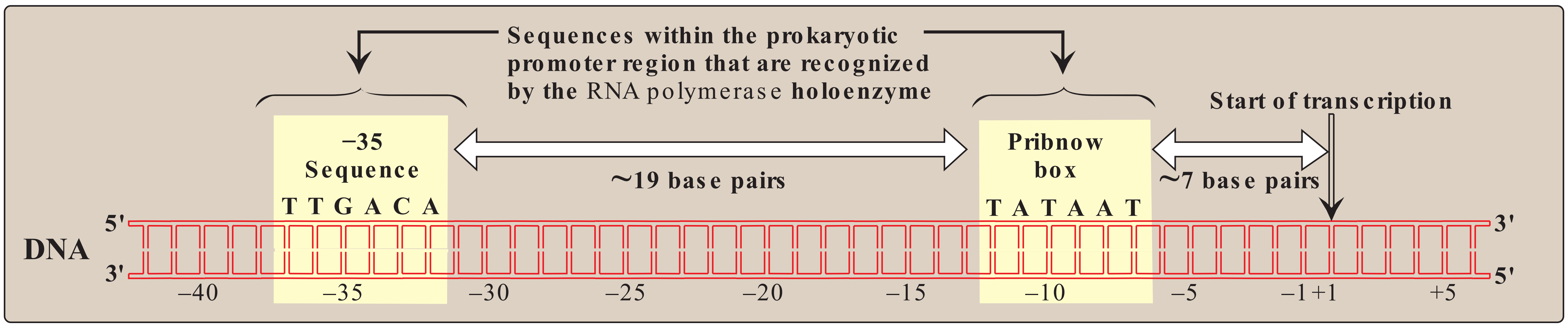

Sequences within the prokaryotic promoter region that are recognized by the RNA polymerase holoenzyme
Start of transcription
−35 Sequence
T T G A C A
~19 base pairs
Pribnow box
T A T A A T
~7 base pairs
DNA
5'
3'
3'
5'
−40
−35
−30
−25
−20
−15
−10
−5
−1 +1
+5

Figure 30.7
Structure of the prokaryotic promoter region. T = thymine; G = guanine; A = adenine; C = cytosine.

at each position is the base most frequently (but not necessarily always) encountered at that position.] Those that are recognized by prokaryotic RNA polymerase s factors include:

a. **–35 Sequence:** A consensus sequence (5'-TTGACA-3'), centered about 35 bases to the left of the transcription start site (see Figure 30.7), is the initial point of contact for the holoenzyme, and a closed complex is formed. [Note: The regulatory sequences that control transcription are, by convention, designated by the 5'→3' nucleotide sequence on the coding strand. A base in the promoter region is assigned a negative number if it occurs prior to (to the left of, toward the 5'-end of, or "upstream" of) the transcription start site. Therefore, the TTGACA sequence is centered at approximately base –35. The first base at the transcription start site is assigned a position of +1. There is no base designated "0".]

b. **Pribnow box:** The holoenzyme moves and covers a second consensus sequence (5'-TATAAT-3'), centered at about –10 (see Figure 30.7), which is the site of initial DNA melting (unwinding). Melting of a short stretch (about 14 bases) converts the closed complex to an open complex known as a transcription bubble. [Note: A mutation in either the –10 or the –35 sequence can affect the transcription of the gene controlled by the mutant promoter.]

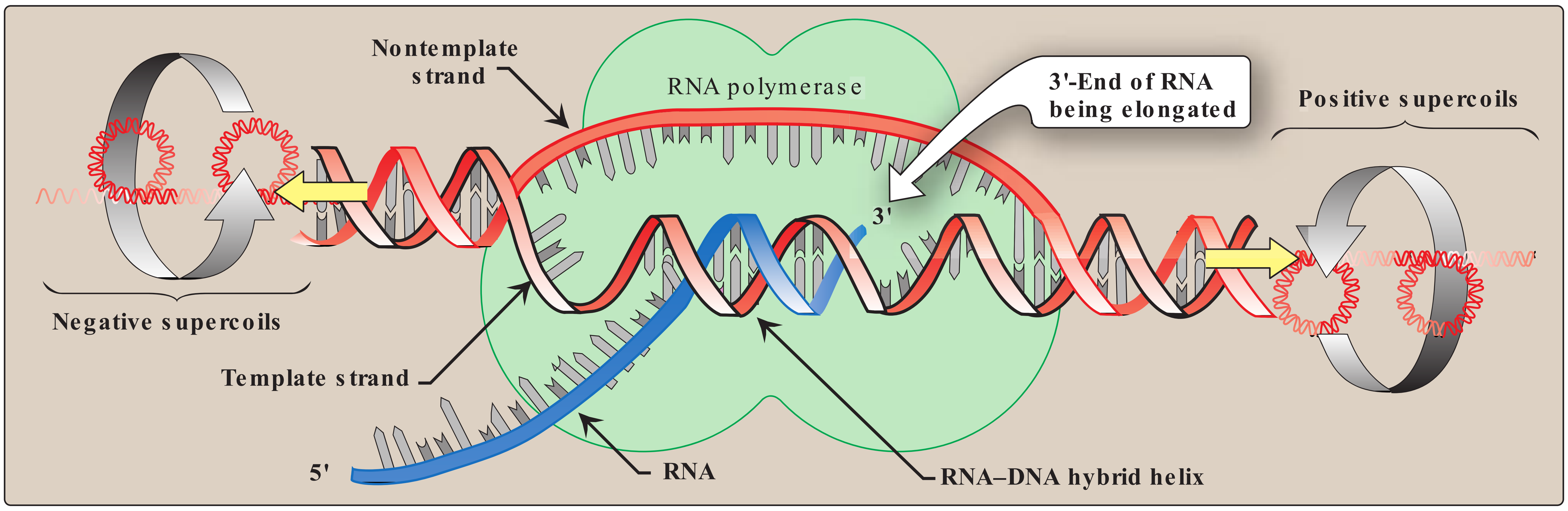

Nontemplate strand
RNA polymerase
3'-End of RNA being elongated
Positive supercoils
3'
Negative supercoils
Template strand
5'
RNA
RNA–DNA hybrid helix

Figure 30.8
Local unwinding of DNA caused by RNA polymerase and formation of an open initiation complex.

2. **Elongation:** Once the promoter region has been recognized and bound by the holoenzyme, local unwinding of the DNA helix continues (Figure 30.8), mediated by the polymerase. [Note: Unwinding generates supercoils in the DNA that can be relieved by DNA topoisomerases (see p. 401.] RNA pol begins to synthesize a transcript of the DNA sequence, and several short pieces of RNA are made and discarded. The elongation phase is said to begin when the transcript (typically starting with a purine) exceeds ten nucleotides in length. Sigma is then released, and the core enzyme is able to leave ("clear") the promoter and move along the template strand in a processive manner, serving as its own sliding clamp. During transcription, a short DNA–RNA hybrid helix is formed (see Figure 30.8). Like DNA pol, RNA pol uses nucleoside triphosphates as substrates and releases pyrophosphate each time a nucleoside monophosphate is added to the growing chain. As with replication, transcription is always in the 5'→3' direction. In contrast to DNA pol, RNA pol does not require a primer and does not appear to have 3'→5' exonuclease (proofreading) activity.

3. **Termination:** The elongation of the single-stranded RNA chain continues until a termination signal is reached. Termination can be intrinsic (spontaneous) or dependent upon the participation of a protein known as the r (rho) factor.

   a. **r-Independent termination**: Seen with most prokaryotic genes, this requires that a sequence in the DNA template generates a sequence in the nascent (newly made) RNA that is self-complementary (Figure 30.9). This allows the RNA to fold back on itself, forming a GC-rich stem (stabilized by hydrogen bonds) plus a loop. This structure is known as a "hairpin." Additionally, just beyond the hairpin, the RNA transcript contains a string of Us at the 3'-end. The bonding of these Us to the complementary As of the DNA template is weak. This facilitates the separation of the newly synthesized RNA from its DNA template, as the double helix "zips up" behind the RNA polymerase.

   b. **r-Dependent termination:** This requires the participation of an additional protein, rho (r), which is a hexameric ATPase with helicase activity. Rho binds a C-rich "rho recognition site" near the 5'-end of the nascent RNA and, using its ATPase activity, moves along the RNA until it reaches the RNA pol paused at the termination site. The ATP-dependent helicase activity of r separates the RNA–DNA hybrid helix, causing the release of the RNA.

4. **Action of antibiotics:** Some antibiotics prevent bacterial cell growth by inhibiting RNA synthesis. For example, rifampin (rifampicin) inhibits transcription by binding to the b subunit of prokaryotic RNA pol, and preventing chain extension beyond three nucleotides (Figure 30.10). Rifampin is important in the treatment of tuberculosis. Dactinomycin (known to biochemists as actinomycin D) was the first antibiotic to find therapeutic application in tumor chemotherapy. It binds to the DNA template and interferes with the movement of RNA pol along the DNA.

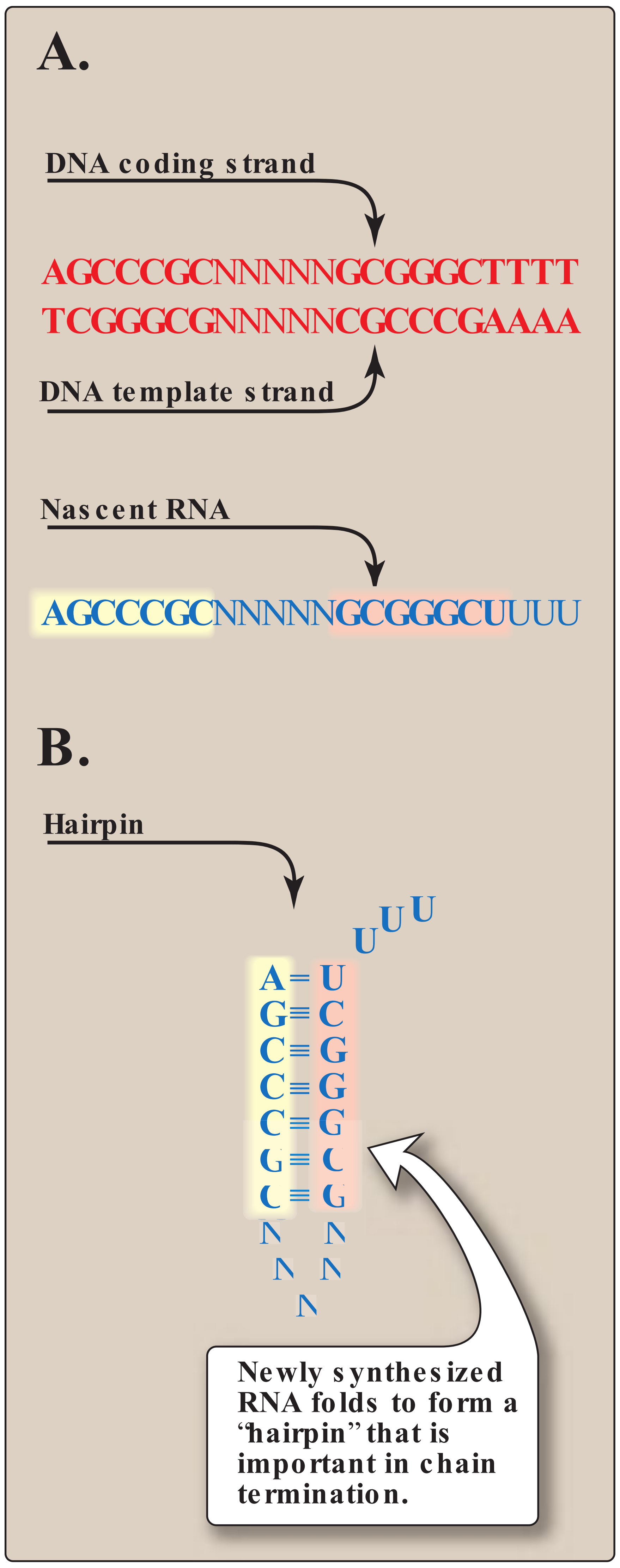
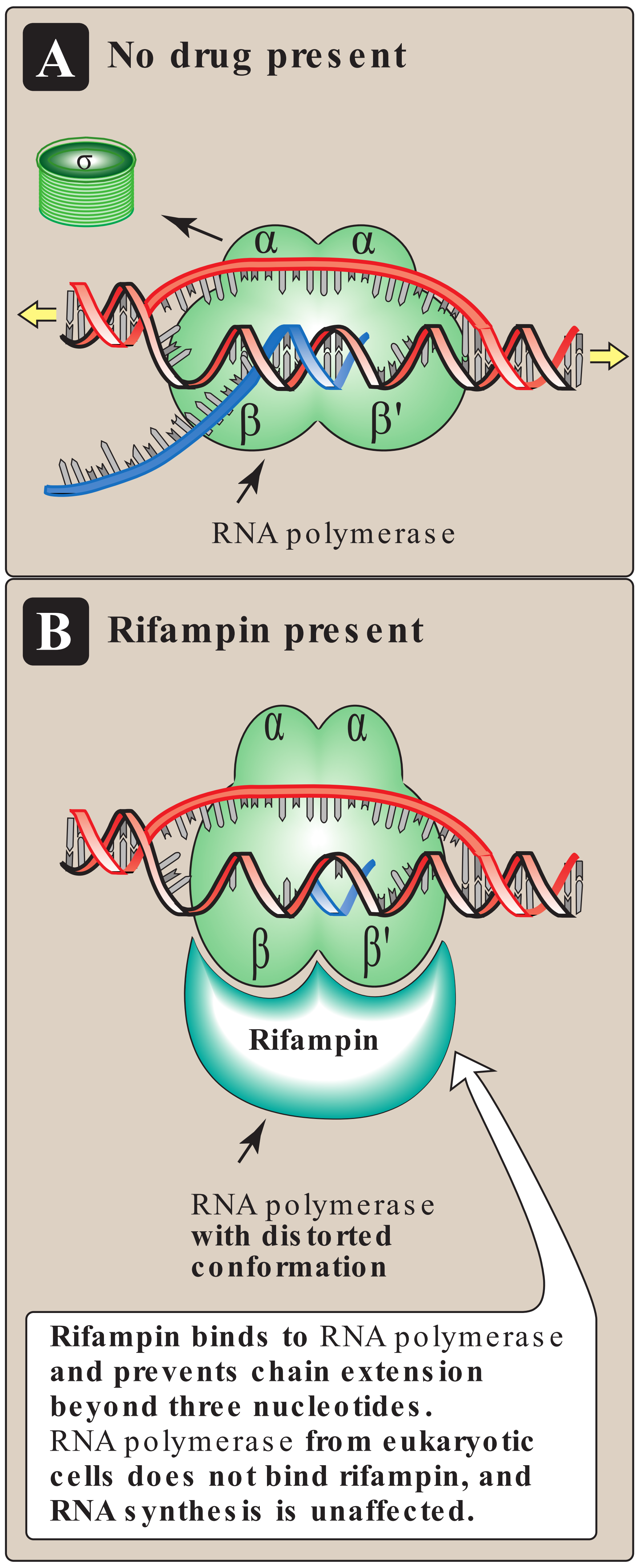


Figure 30.9
Rho-independent termination of prokaryotic transcription. A. DNA template sequence generates a self-complementary sequence in the nascent RNA. B. Hairpin structure formed by the RNA. "N" represents a noncomplementary base; A = adenine, T = thymine; G = guanine; C = cytosine; U = uracil. [Note: Termination of eukaryotic transcription is not well understood.]



Rifampin binds to RNA polymerase **and prevents chain extension beyond three nucleotides.**
RNA polymerase **from eukaryotic cells does not bind rifampin, and RNA synthesis is unaffected.**

Figure 30.10
Inhibition of prokaryotic RNA polymerase by rifampin.

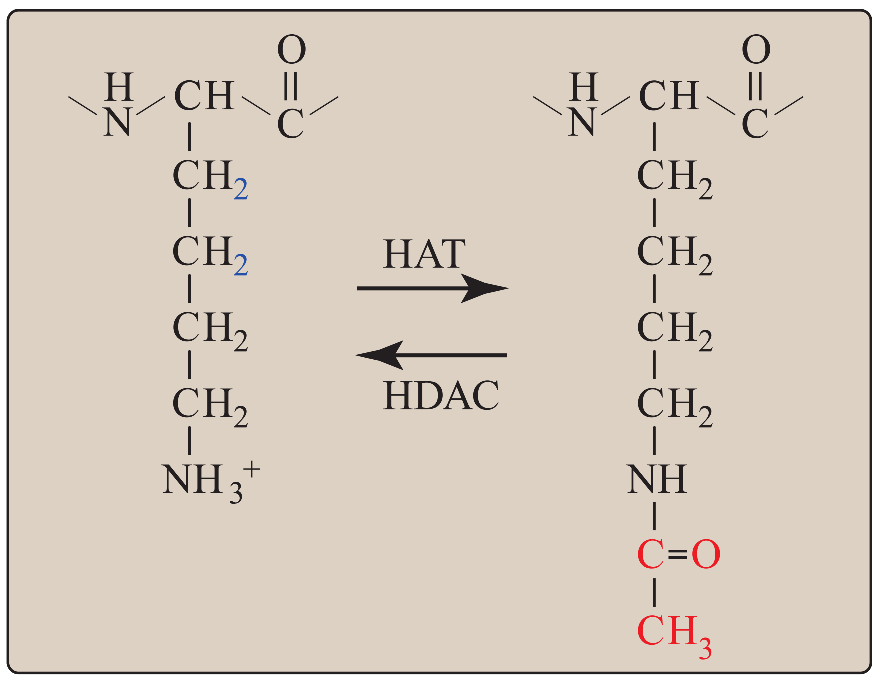


Figure 30.11
Acetylation/deacetylation of a lysine residue in a histone protein. HAT = histone acetyltransferase; HDAC = histone deacetylase.

IV. TRANSCRIPTION OF EUKARYOTIC GENES

The transcription of eukaryotic genes is a far more complicated process than transcription in prokaryotes. Eukaryotic transcription involves separate polymerases for the synthesis of rRNA, tRNA, and mRNA. In addition, a large number of proteins called transcription factors (TFs) are involved. TFs bind to distinct sites on the DNA either within the core promoter region, close (proximal) to it, or some distance away (distal). They are required both for the assembly of a transcription complex at the promoter and the determination of which genes are to be transcribed. [Note: Each eukaryotic RNA pol has its own promoters and TFs that bind core promoter sequences.] For TFs to recognize and bind to their specific DNA sequences, the chromatin structure in that region must be altered (relaxed) to allow access to the DNA. The role of transcription in the regulation of gene expression is discussed in Chapter 32.

A. Chromatin structure and gene expression

The association of DNA with histones to form nucleosomes (see p. 409) affects the ability of the transcription machinery to access the DNA to be transcribed. Most actively transcribed genes are found in a relatively relaxed form of chromatin called euchromatin, whereas most inactive segments of DNA are found in highly condensed heterochromatin. [Note: The interconversion of these forms is called chromatin remodeling.] A major component of chromatin remodeling is the covalent modification of histones (for example, the acetylation of lysine residues at the amino terminus of histone proteins) as shown in Figure 30.11. Acetylation, mediated by histone acetyltransferases (HATs), eliminates the positive charge on the lysine, thereby decreasing the interaction of the histone with the negatively charged DNA. Removal of the acetyl group by histone deacetylases (HDACs) restores the positive charge and fosters stronger interactions between histones and DNA. [Note: The ATP-dependent repositioning of nucleosomes is also required to access DNA.]

B. Nuclear RNA polymerases of eukaryotic cells

There are three distinct classes of RNA pol in the nucleus of eukaryotic cells. All are large enzymes with multiple subunits. Each class of RNA pol recognizes particular types of genes.

1. **RNA polymerase I:** This enzyme synthesizes the precursor of the 28S, 18S, and 5.8S rRNA in the nucleolus.
2. **RNA polymerase II:** This enzyme synthesizes the nuclear precursors of mRNA that are subsequently translated to produce proteins. RNA pol II also synthesizes certain small ncRNAs, such as snoRNA (see p. 425), snRNA (see p. 426) and miRNA (see p. 459).

   a. **Promoters for RNA polymerase II:** In some genes transcribed by RNA pol II, a sequence of nucleotides (TATAAA) that is nearly identical to that of the Pribnow box (see p. 420) is found centered about 25 nucleotides upstream of the transcription start site. This core promoter consensus sequence is called the TATA, or Hogness, box. In the majority of genes, however, no TATA box is present. Instead, different core promoter elements

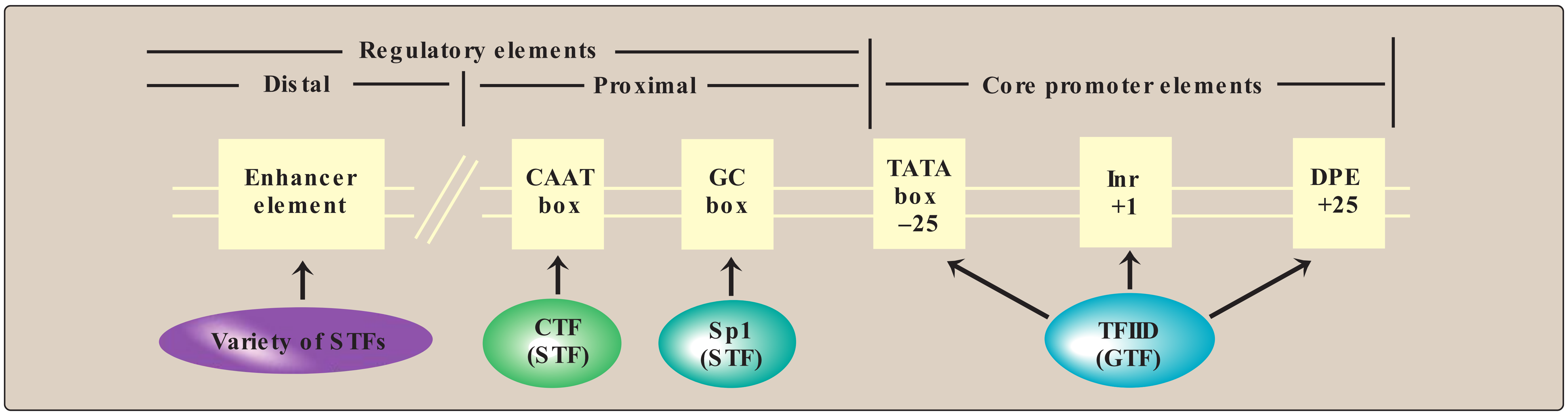

Regulatory elements
Distal
Proximal
Core promoter elements
Enhancer element
CAAT box
GC box
TATA box –25
Inr +1
DPE +25
Variety of STFs
CTF (STF)
Sp1 (STF)
TFIID (GTF)

Figure 30.12
Eukaryotic gene cis-acting promoter and regulatory elements and their trans-acting general and specific transcription factors (GTF and STF, respectively). Inr = initiator; DPE = downstream promoter element.

such as Inr (initiator) or DPE (downstream promoter element) are present (Figure 30.12). [Note: No one consensus sequence is found in all core promoters.] Because these sequences are on the same molecule of DNA as the gene being transcribed, they are cis-acting. The sequences serve as binding sites for proteins known as general transcription factors (GTFs), which in turn interact with each other and with RNA pol II.

b. **General transcription factors:** These are the minimal requirements for recognition of the promoter, recruitment of RNA pol II to the promoter, and initiation of transcription at a basal level (Figure 30.13A). GTFs are encoded by different genes, synthesized in the cytosol, and transit to their sites of action, and so are trans-acting. [Note: In contrast to the prokaryotic holoenzyme, eukaryotic RNA pol II does not itself recognize and bind the promoter. Instead, TFIID, a GTF containing TATA-binding protein and TATA-associated factors, recognizes and binds the TATA box (and other core promoter elements). TFIIF, another GTF, brings the polymerase to the promoter. The helicase activity of TFIIH melts the DNA, and its kinase activity phosphorylates polymerase, allowing it to clear the promoter.]

c. **Regulatory elements and transcriptional activators:** Upstream of the core promoter are additional consensus sequences (see Figure 30.12). Those close to the core promoter (within 200 nucleotides) are the proximal regulatory elements, such as the CAAT and GC boxes. Those farther away are the distal regulatory elements such as enhancers (see p. 424). Proteins known as transcriptional activators or specific transcription factors (STFs) bind these regulatory elements. STFs bind to promoter proximal elements to regulate the frequency of transcription initiation, and to distal elements to mediate the response to signals such as hormones (see p. 456) and regulate which genes are expressed at a given point in time. A typical protein-coding eukaryotic gene has binding sites for many such factors. [Note: STFs have two binding domains. One is a DNA-binding domain, the other is a transcription activation domain that recruits the GTFs to the core promoter as well as "coactivator" proteins such as the HAT enzymes involved in chromatin modification.]

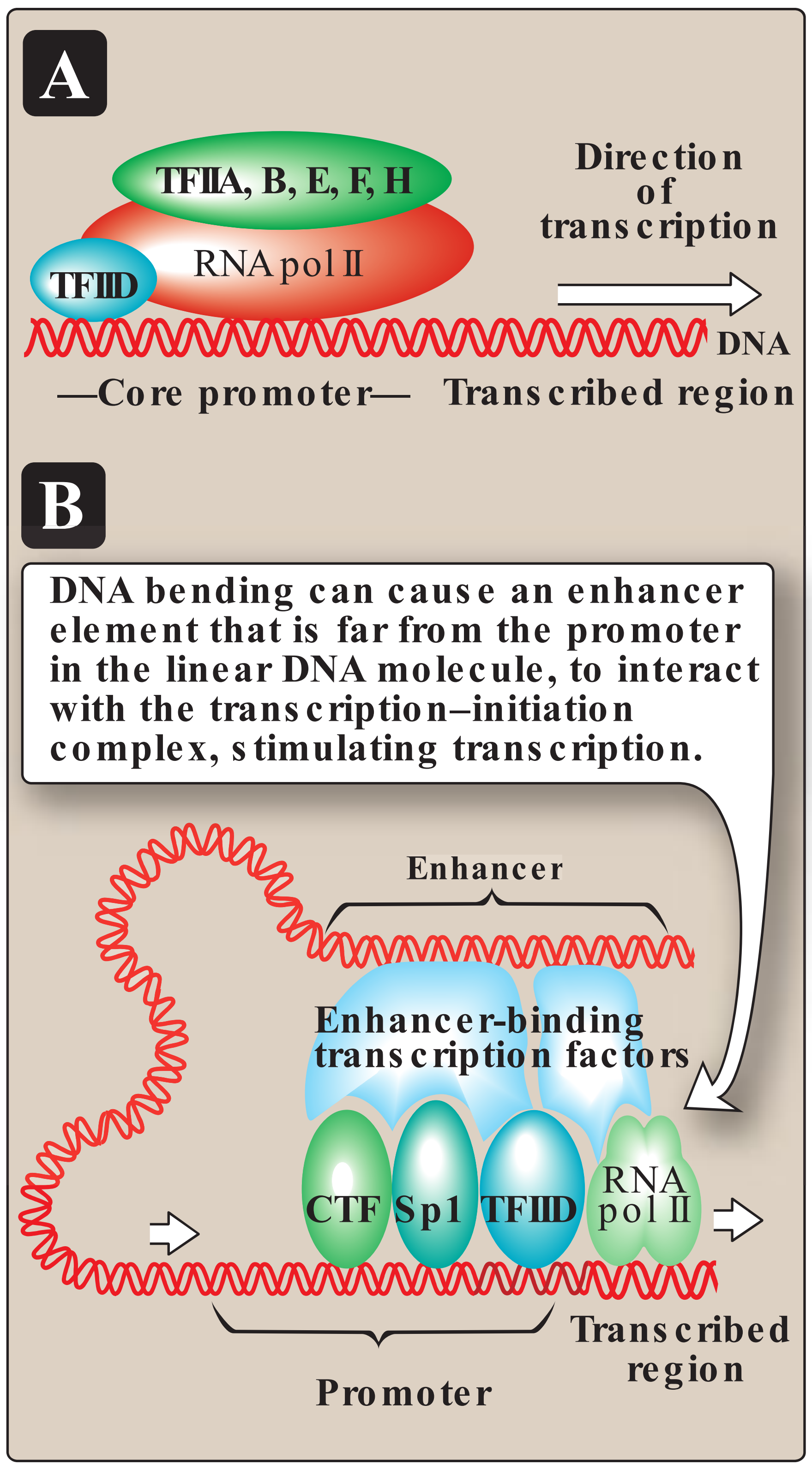

A
TFIIA, B, E, F, H
RNA pol II
TFIID
Direction of transcription
DNA
—Core promoter—
Transcribed region
B
DNA bending can cause an enhancer element that is far from the promoter in the linear DNA molecule, to interact with the transcription–initiation complex, stimulating transcription.
Enhancer
Enhancer-binding transcription factors
CTF
Sp1
TFIID
RNA pol II
Transcribed region
Promoter

Figure 30.13
A. Association of the general transcription factors (TFIIs) and RNA polymerase II (RNA pol II) at the core promoters. [Note: The Roman numeral II denotes the TFs for RNA pol II.] B. Enhancer stimulation of transcription. CTF = CAAT box transcription factor; Sp1 = specificity factor-1.

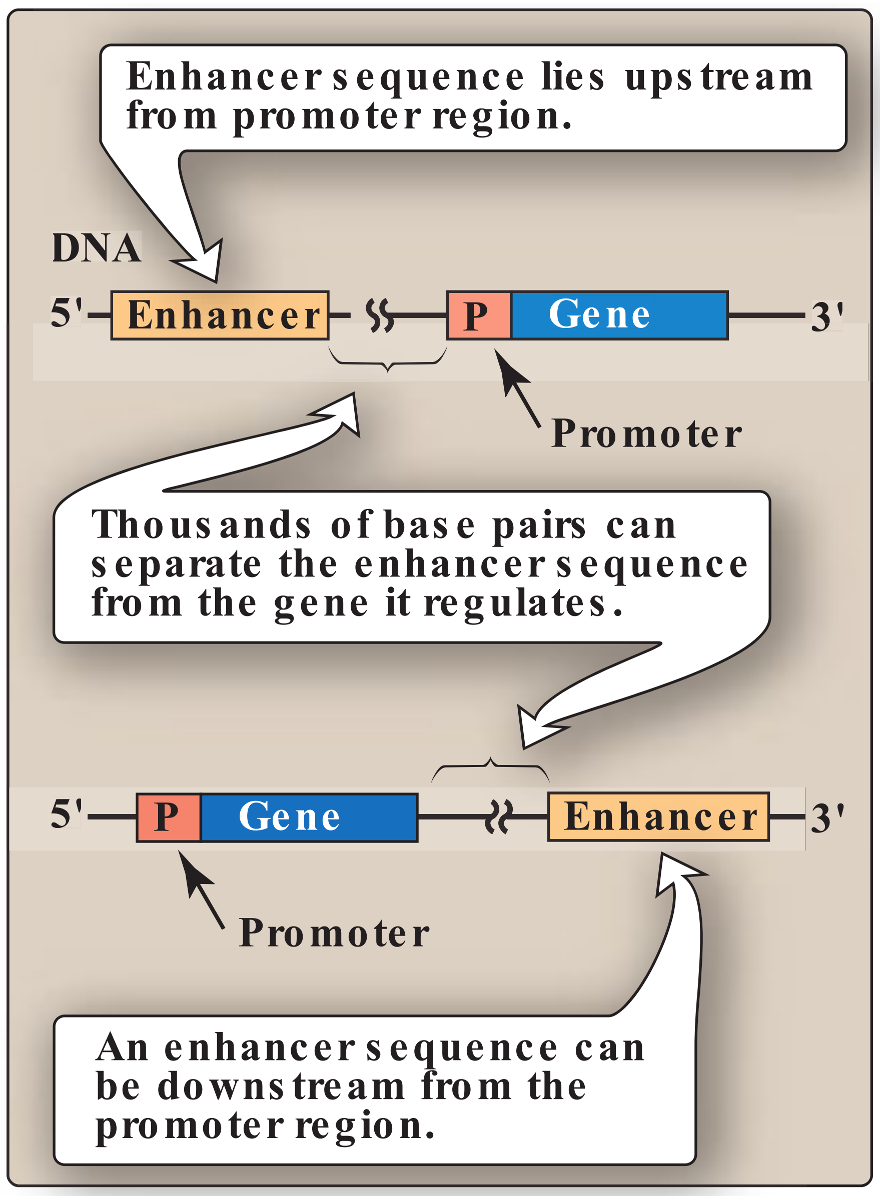

Enhancer sequence lies upstream from promoter region.
DNA
5'
Enhancer
P
Gene
3'
Promoter
Thousands of base pairs can separate the enhancer sequence from the gene it regulates.
5'
P
Gene
Enhancer
3'
Promoter
An enhancer sequence can be downstream from the promoter region.

Figure 30.14
Some possible locations of enhancer sequences.

> Transcriptional activators bind DNA through a variety of motifs, such as the helix-loop-helix, zinc finger, and leucine zipper (see p. 18).

d. **Role of enhancers in eukaryotic gene regulation:** Enhancers are special DNA sequences that increase the rate of initiation of transcription by RNA pol II. Enhancers are typically on the same chromosome as the gene whose transcription they stimulate (Figure 30.13B). However, they can 1) be located upstream (to the 5'-side) or downstream (to the 3'-side) of the transcription start site, 2) be close to or thousands of base pairs away from the promoter (Figure 30.14), and 3) occur on either strand of the DNA. Enhancers contain DNA sequences called "response elements" that bind STFs (transcriptional activators). By bending or looping the DNA, these enhancer-binding proteins can interact with other transcription factors bound to a promoter and with RNA pol II, thereby stimulating transcription (see Figure 30.13B). [Note: Although silencers are similar to enhancers in that they also can act over long distances, they reduce gene expression.]

e. **Inhibitors of RNA polymerase II:** α-Amanitin, a potent toxin produced by the poisonous mushroom Amanita phalloides (sometimes called "the death cap"), forms a tight complex with RNA pol II, thereby inhibiting mRNA synthesis.

3. **RNA polymerase III:** This enzyme synthesizes tRNA, 5S rRNA, and some snRNA and snoRNA.

C. Mitochondrial RNA polymerase

Mitochondria contain a single RNA pol that more closely resembles bacterial RNA pol than the eukaryotic nuclear enzymes.

V. POSTTRANSCRIPTIONAL MODIFICATION OF RNA

A primary transcript is the initial, linear, RNA copy of a transcription unit (the segment of DNA between specific initiation and termination sequences). The primary transcripts of both prokaryotic and eukaryotic tRNA and rRNA are posttranscriptionally modified by cleavage of the original transcripts by ribonucleases. tRNAs are then further modified to help give each species its unique identity. In contrast, prokaryotic mRNA is generally identical to its primary transcript, whereas eukaryotic mRNA is extensively modified both co- and posttranscriptionally.

A. Ribosomal RNA

rRNAs of both prokaryotic and eukaryotic cells are generated from long precursor molecules called pre-rRNAs. The 23S, 16S, and 5S rRNA of prokaryotes are produced from a single pre-rRNA molecule, as are the 28S, 18S, and 5.8S rRNA of eukaryotes (Figure 30.15). [Note: Eukaryotic 5S rRNA is synthesized by RNA pol III and modified separately.] The pre-rRNAs are cleaved by ribonucleases to yield intermediate-sized pieces of rRNA, which are further processed (trimmed by exonucleases and modified at some bases and riboses)

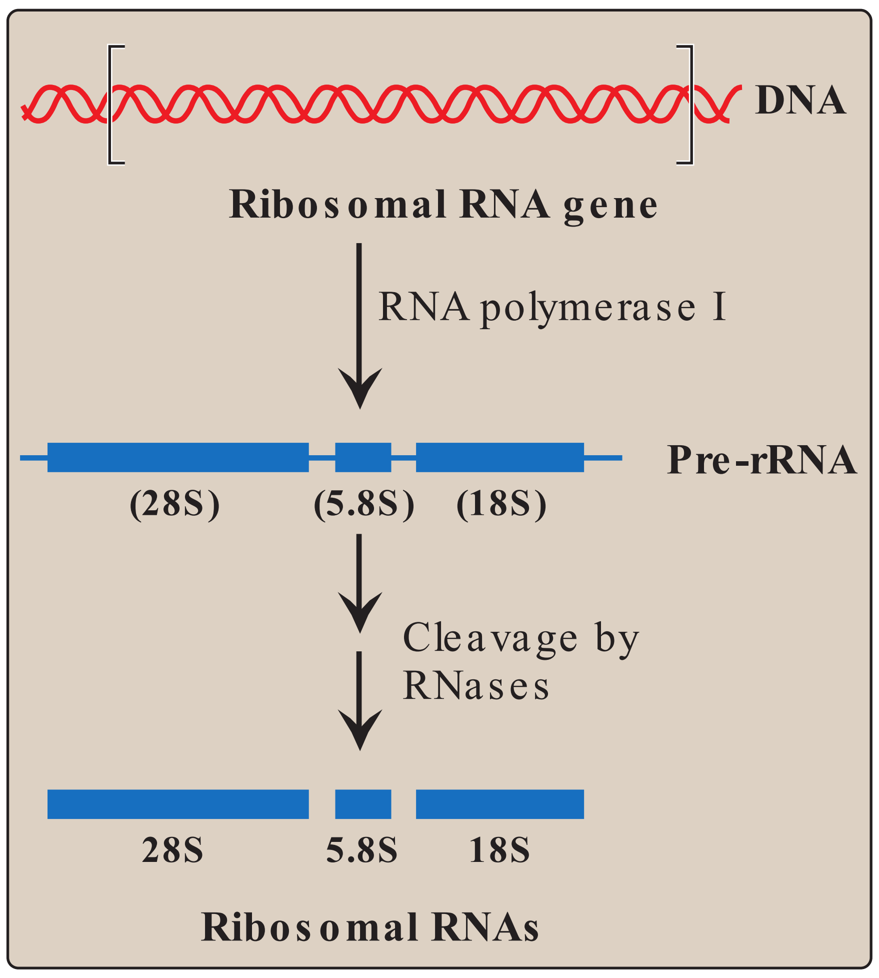

DNA
Ribosomal RNA gene
RNA polymerase I
Pre-rRNA
(28S)
(5.8S)
(18S)
Cleavage by RNases
28S
5.8S
18S
Ribosomal RNAs

Figure 30.15
Posttranscriptional processing of eukaryotic ribosomal RNA by ribonucleases (RNases).
S = Svedberg unit.

to produce the required RNA species. [Note: In eukaryotes, rRNA genes are found in long, tandem arrays. rRNA synthesis and processing occur in the nucleolus, with base and sugar modifications facilitated by snoRNA. Some of the proteins destined to become components of the ribosome associate with pre-rRNA prior to and during its modification.]

B. Transfer RNA

Both eukaryotic and prokaryotic tRNAs are also made from longer precursor molecules that must be modified (Figure 30.16). Sequences at both ends of the molecule are removed, and, if present, an intron is removed from the anticodon loop by nucleases. Other posttranscriptional modifications include addition of a –CCA sequence by nucleotidyltransferase to the 3'-terminal end of tRNA, and modification of bases at specific positions to produce the “unusual bases” characteristic of tRNA (see p. 292).

C. Eukaryotic mRNA

The collection of all the primary transcripts synthesized in the nucleus by RNA pol II is known as heterogeneous nuclear RNA (hnRNA). The pre-mRNA components of hnRNA undergo extensive co- and posttranscriptional modification in the nucleus. These modifications usually include the following.

1. **5' “Capping”:** This is the first of the processing reactions for pre-mRNA (Figure 30.17). The cap is a 7-methylguanosine attached to the 5'-terminal end of the mRNA through an unusual 5'→5' triphosphate linkage that is resistant to most nucleases. Creation of the cap requires removal of the g phosphoryl group from the 5'-triphosphate of the pre-mRNA, followed by addition of guanosine

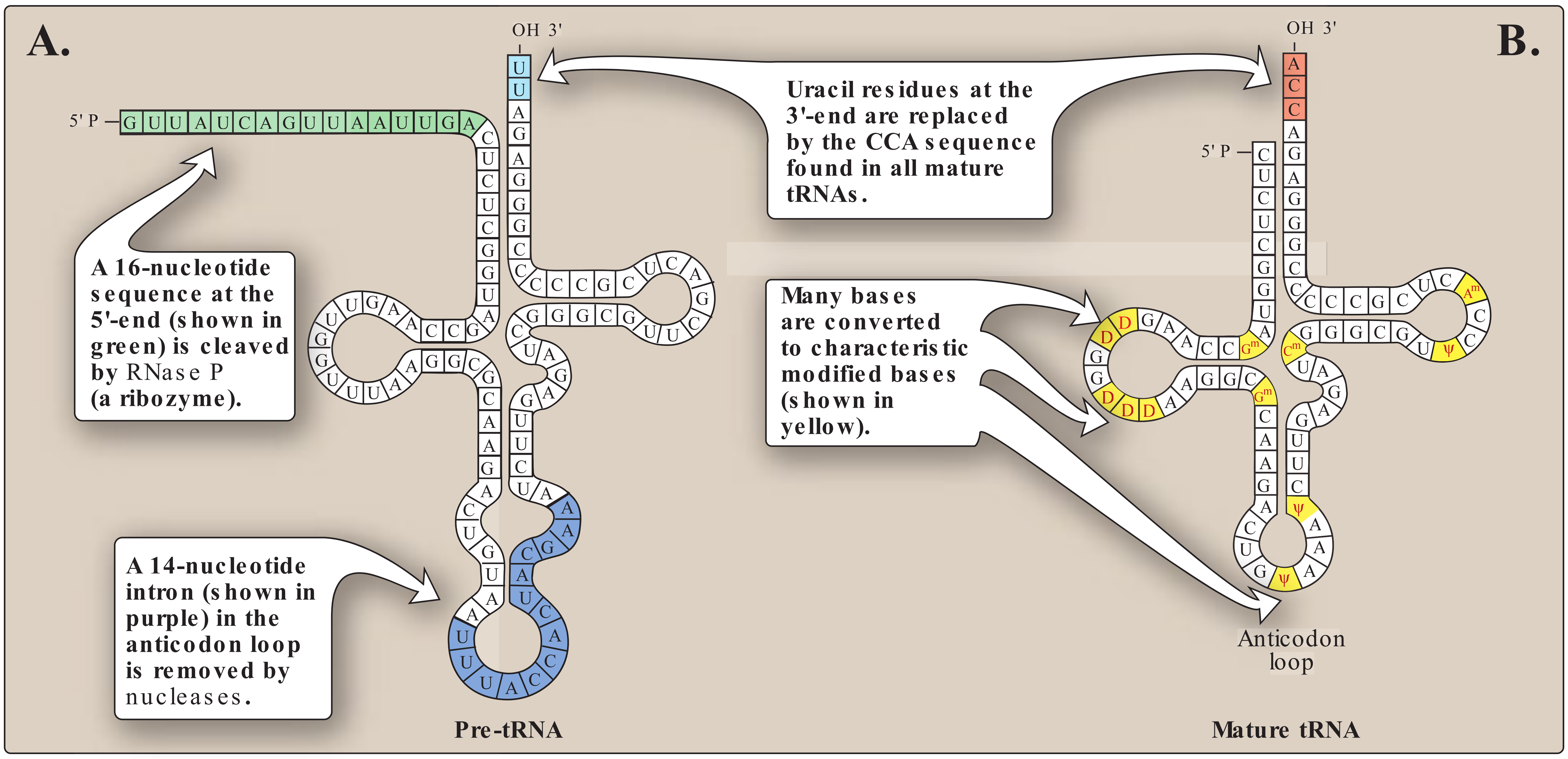

A.
B.
OH 3'
5' P
A 16-nucleotide sequence at the 5'-end (shown in green) is cleaved by RNase P (a ribozyme).
Uracil residues at the 3'-end are replaced by the CCA sequence found in all mature tRNAs.
Many bases are converted to characteristic modified bases (shown in yellow).
A 14-nucleotide intron (shown in purple) in the anticodon loop is removed by nucleases.
Anticodon loop
Pre-tRNA
Mature tRNA

Figure 30.16
A. Primary transfer RNA (tRNA) transcript. B. Functional tRNA after posttranscriptional modification. Modified bases include D (dihydrouracil); ψ (pseudouracil); and ^{m}, which means that the base has been methylated.

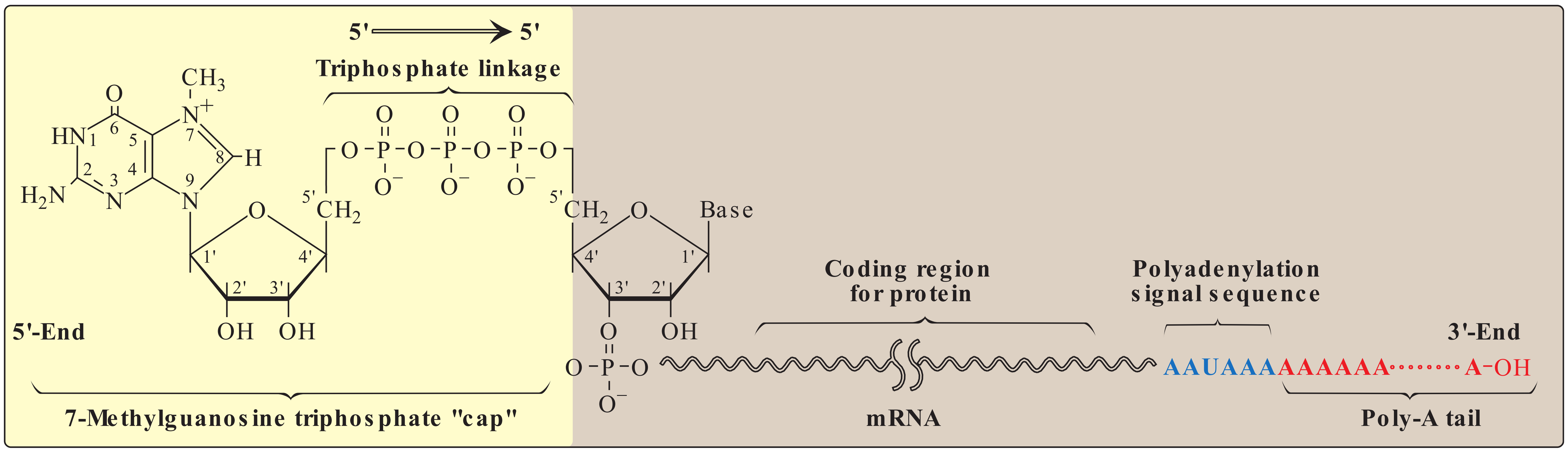

5' → 5'
Triphosphate linkage
5'-End
7-Methylguanosine triphosphate "cap"
Base
Coding region for protein
Polyadenylation signal sequence
3'-End
AAUAAAAAAAAA·······A-OH
mRNA
Poly-A tail

Figure 30.17
Posttranscriptional modification of messenger RNA (mRNA) showing the 7-methylguanosine cap and poly-A tail.

monophosphate (GMP) (from GTP) by the nuclear enzyme guanylyltransferase. Methylation of this terminal guanine occurs in the cytosol and is catalyzed by guanine-7-methyltransferase. S-adenosylmethionine is the source of the methyl group (see p. 263). Additional methylation steps may occur. The addition of this 7-methylguanosine cap helps stabilize the mRNA and permits efficient initiation of translation (see p. 439).

2. **Addition of a poly-A tail:** Most eukaryotic mRNA (with several notable exceptions, including those coding for the histones) have a chain of 40–250 adenine nucleotides attached to the 3'-end (see Figure 30.17). This poly-A tail is not transcribed from the DNA, but rather is added after transcription by the nuclear enzyme, polyadenylate polymerase, using ATP as the substrate. The pre-mRNA is cleaved downstream of a consensus sequence, called the polyadenylation signal sequence (AAUAAA), found near the 3'-end of the RNA, and the poly-A tail is added to the new 3'-end. These tails help stabilize the mRNA, facilitate its exit from the nucleus, and aid in translation. After the mRNA enters the cytosol, the poly-A tail is gradually shortened.

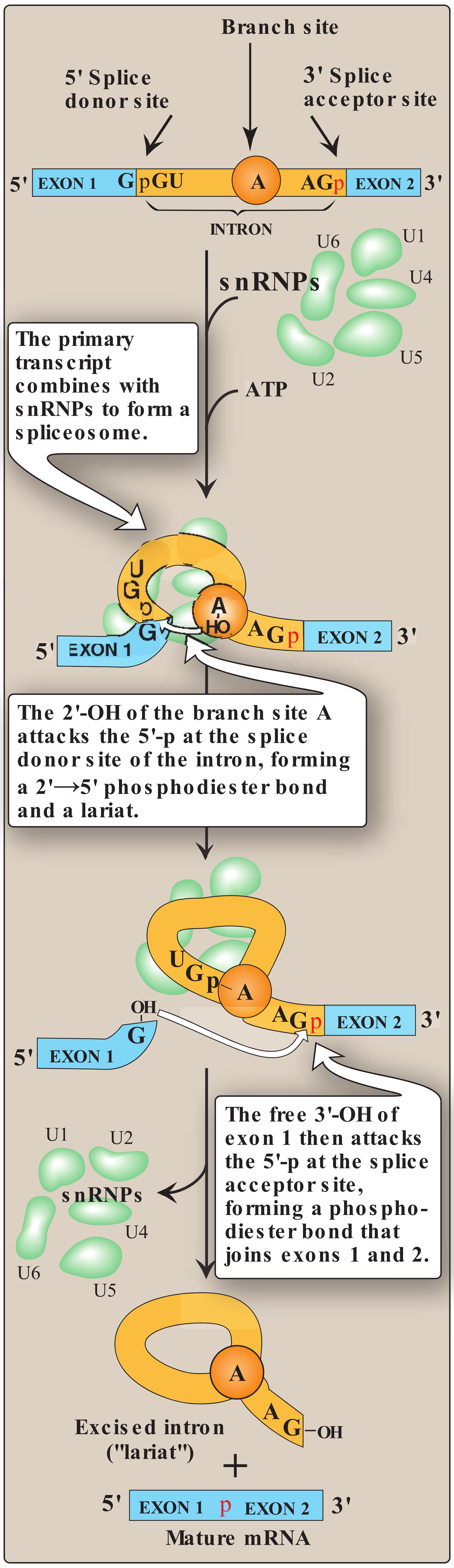
3. **Removal of introns:** Maturation of eukaryotic mRNA usually involves removal from the primary transcript of RNA sequences (introns, or intervening sequences) that do not code for protein. The remaining coding (expressed) sequences, the exons, are joined together to form the mature mRNA. The process of removing introns and joining exons is called splicing. The molecular complex that accomplishes these tasks is known as the spliceosome. A few eukaryotic primary transcripts contain no introns (for example, those from histone genes). Others contain a few introns, whereas some, such as the primary transcripts for the α chains of collagen, contain more than 50 intervening sequences that must be removed before mature mRNA is ready for translation.

   a. **Role of small nuclear RNAs:** In association with multiple proteins, uracil-rich snRNAs form small nuclear ribonucleoprotein particles (snRNPs, or “snurps,” designated as U1, U2, U4, U5, and U6) that mediate splicing. They facilitate the removal of introns by forming base pairs with the consensus sequences at each end of the intron (Figure 30.18). [Note: In systemic lupus erythematosus, an autoimmune disease, patients produce antibodies against their own nuclear proteins such as snRNPs.]

b. Mechanism of splicing: The binding of snRNPs brings the sequences of the neighboring exons into the correct alignment for splicing, allowing two transesterification reactions to occur. The 2'-OH group of an adenine nucleotide (known as the branch site A) in the intron attacks the phosphate at the 5'-end of the intron (splice donor site), forming an unusual 2'→5' phosphodiester bond and creating a "lariat" structure (see Figure 30.18). The newly freed 3'-OH of exon 1 attacks the 5'-phosphate at the splice acceptor site, forming a phosphodiester bond that joins exons 1 and 2. The excised intron is released as a lariat, which is typically degraded. [Note: The GU and AG sequences at the beginning and end, respectively, of introns are invariant.] After introns have been removed and exons joined, the mature mRNA molecules leave the nucleus and pass into the cytosol through pores in the nuclear membrane. [Note: The introns in tRNA (see Figure 30.16) are removed by a different mechanism.]

c. Effect of splice site mutations: Mutations at splice sites can lead to improper splicing and the production of aberrant proteins. It is estimated that over 15% of all genetic diseases are a result of mutations that affect RNA splicing. For example, mutations that cause the incorrect splicing of b-globin mRNA are responsible for some cases of b-thalassemia, a disease in which the production of the b-globin protein is defective (see p. 38). Splice site mutations can result in exons being skipped (removed) or introns retained. They can also activate cryptic splice sites, which are sites that contain the 5' or 3' consensus sequence but aren't normally used.

4. **Alternative splicing of mRNA molecules:** The pre-mRNA molecules from over 50% of human genes can be spliced in alternative ways in different tissues. This produces multiple variations of the mRNA and, therefore, of its protein product (Figure 30.19), and thus is a mechanism for producing a large, diverse set of proteins from a limited set of genes. For example, in eukaryotic cells, the mRNA for tropomyosin, an actin filament–binding protein of the cytoskeleton (and of the contractile apparatus in muscle cells), undergoes extensive tissue-specific alternative splicing with production of multiple isoforms of the tropomyosin protein.



The primary transcript combines with snRNPs to form a spliceosome.

The 2'-OH of the branch site A attacks the 5'-p at the splice donor site of the intron, forming a 2'→5' phosphodiester bond and a lariat.

The free 3'-OH of exon 1 then attacks the 5'-p at the splice acceptor site, forming a phosphodiester bond that joins exons 1 and 2.

Figure 30.18
Splicing. snRNP = small nuclear ribonucleoprotein particle; mRNA = messenger RNA. [Note: U1 binds the 5' donor site, U2 binds the branch A, and addition of U4–U6 completes the complex.]

VI. CHAPTER SUMMARY

There are three major types of RNA that participate in the process of protein synthesis: **ribosomal RNA (rRNA)**, **transfer RNA (tRNA)**, and **messenger RNA (mRNA)** (Figure 30.20). They are unbranched polymers of nucleotides but differ from DNA by containing **ribose** instead of deoxyribose and **uracil** instead of thymine. **rRNA** is a component of the **ribosomes**. **tRNA** serves as an "adaptor" molecule that carries a

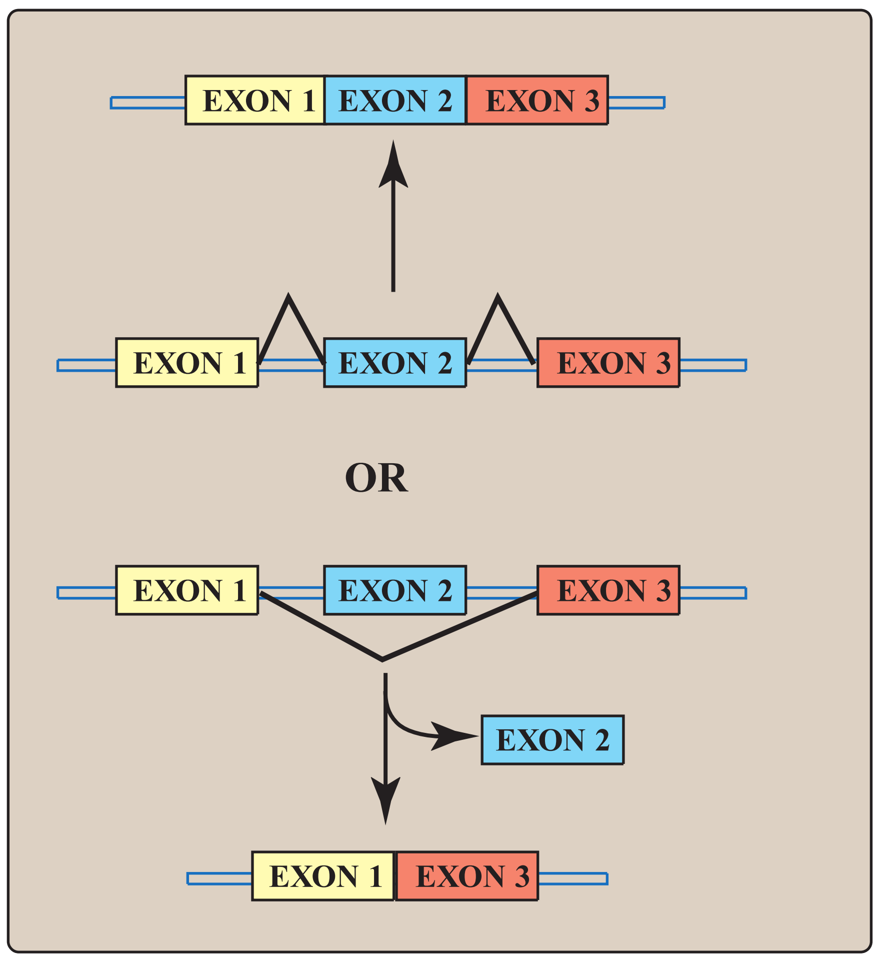


Figure 30.19
Alternative splicing patterns in eukaryotic messenger RNA.

specific amino acid to the site of protein synthesis. **mRNA** carries genetic information from DNA for use in protein synthesis. The process of RNA synthesis is called **transcription**, and the substrates are **ribonucleoside triphosphates**. The enzyme that synthesizes RNA is RNA polymerase (RNA pol). In **prokaryotic** cells, the **core enzyme** has five subunits (2α, 1b, 1b', and 1W) and possesses 5'→3' polymerase **activity** that **elongates** the growing RNA strand. This enzyme requires an additional subunit, sigma (**s**) **factor**, that recognizes the nucleotide sequence (**promoter** region) at the beginning of a length of DNA that is to be transcribed. This region contains **consensus sequences** that are highly conserved and include the **TATA** (**Pribnow**) **box** and the **–35 sequence**. Another protein—rho (**r**) **factor**—is required for **termination** of transcription of some genes. There are three distinct classes of RNA pol in the nucleus of eukaryotic cells. RNA pol I synthesizes the precursor of rRNAs in the nucleolus. In the nucleoplasm, RNA pol II synthesizes the precursors for mRNA and some noncoding RNAs, and RNA pol III produces the precursors of **tRNA**. In both prokaryotes and eukaryotes, RNA pol does not require a primer and has no 3'→5' exonuclease (proofreading) activity. Core **promoters** for genes transcribed by RNA pol II contain cis-acting consensus sequences, such as the TATA-like **Hogness box**, that serve as binding sites for trans-acting general transcription factors. Upstream of these are proximal regulatory elements, such as the CAAT and GC boxes, and distal regulatory elements such as enhancers. Transcriptional activators (specific transcription factors) bind these elements and regulate the frequency of transcription initiation, the response to signals such as hormones, and which genes are expressed at a given point in time. Eukaryotic transcription requires that the **chromatin** be accessible. **A primary transcript** is a linear copy of a **transcription unit**, the segment of DNA between specific initiation and termination sequences. The primary transcripts of both prokaryotic and eukaryotic tRNA and rRNA are **posttranscriptionally modified** by cleavage of the original transcripts by ribonucleases. **rRNAs** of both prokaryotic and eukaryotic cells are synthesized from long precursor molecules called pre-rRNA. These precursors are cleaved and trimmed by ribonucleases, producing the three largest rRNA, and bases and sugars are modified. Eukaryotic 5S rRNA is synthesized by RNA pol III and is modified separately. Prokaryotic mRNA is generally identical to its primary transcript, whereas eukaryotic mRNA is extensively modified co- and posttranscriptionally. For example, a **7-methylguanosine cap** is attached to the 5'-terminal end of the mRNA through a 5'→5' linkage. A **long poly-A tail**, not transcribed from the DNA, is attached to the 3'-end of most mRNAs. Most eukaryotic mRNAs also contain **intervening sequences** (**introns**) that must be removed to make the mRNA functional. Their removal, as well as the **joining of expressed sequences** (**exons**), requires a **spliceosome** composed of **small, nuclear ribonucleoprotein particles** (**snurps**) that mediate the process of **splicing**. Eukaryotic mRNA is monocistronic, containing information from just one gene. Prokaryotic and eukaryotic tRNAs are also made from longer precursor molecules. If present, an intron is removed by nucleases, and both ends of the molecule are trimmed by ribonucleases. A **3'-CCA** sequence is added, and bases at specific positions are modified, producing “unusual” bases.

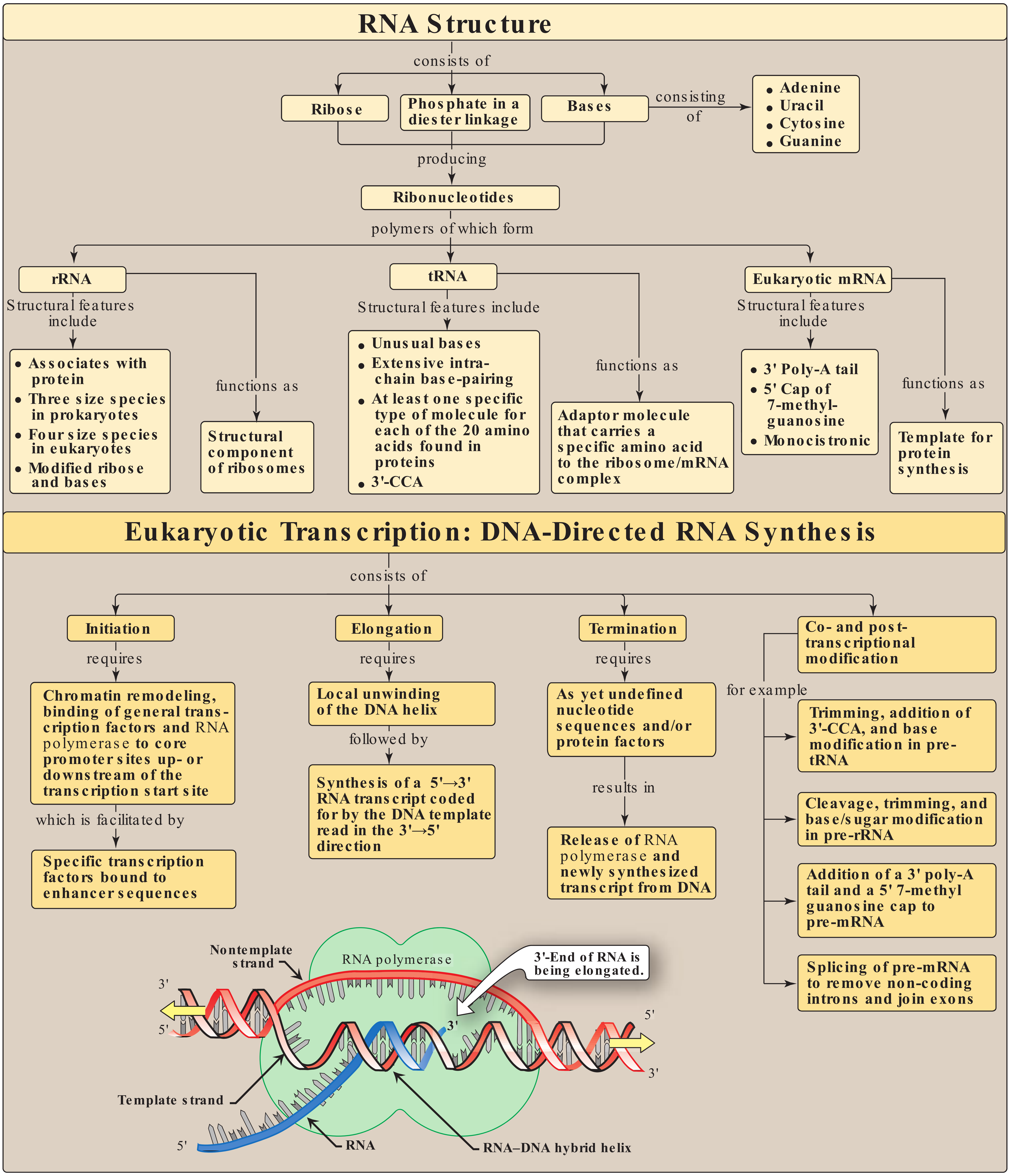

RNA Structure
consists of
Ribose
Phosphate in a diester linkage
Bases
consisting of
• Adenine
• Uracil
• Cytosine
• Guanine
producing
Ribonucleotides
polymers of which form
rRNA
Structural features include
• Associates with protein
• Three size species in prokaryotes
• Four size species in eukaryotes
• Modified ribose and bases
functions as
Structural component of ribosomes
tRNA
Structural features include
• Unusual bases
• Extensive intra-chain base-pairing
• At least one specific type of molecule for each of the 20 amino acids found in proteins
• 3'-CCA
functions as
Adaptor molecule that carries a specific amino acid to the ribosome/mRNA complex
Eukaryotic mRNA
Structural features include
• 3' Poly-A tail
• 5' Cap of 7-methyl-guanosine
• Monocistronic
functions as
Template for protein synthesis
Eukaryotic Transcription: DNA-Directed RNA Synthesis
consists of
Initiation
requires
Chromatin remodeling, binding of general transcription factors and RNA polymerase to core promoter sites up- or downstream of the transcription start site
which is facilitated by
Specific transcription factors bound to enhancer sequences
Elongation
requires
Local unwinding of the DNA helix
followed by
Synthesis of a 5'→3' RNA transcript coded for by the DNA template read in the 3'→5' direction
Termination
requires
As yet undefined nucleotide sequences and/or protein factors
results in
Release of RNA polymerase and newly synthesized transcript from DNA
Co- and posttranscriptional modification
for example
Trimming, addition of 3'-CCA, and base modification in pre-tRNA
Cleavage, trimming, and base/sugar modification in pre-rRNA
Addition of a 3' poly-A tail and a 5' 7-methyl guanosine cap to pre-mRNA
Splicing of pre-mRNA to remove non-coding introns and join exons
Nontemplate strand
RNA polymerase
3'-End of RNA is being elongated.
3'
5'
3'
5'
3'
Template strand
5'
RNA
RNA–DNA hybrid helix

Figure 30.20
Key concept map for RNA structure and synthesis. rRNA = ribosomal RNA; tRNA = transfer RNA; mRNA = messenger RNA.

Protein Synthesis

31

I. OVERVIEW

Genetic information, stored in the chromosomes and transmitted to daughter cells through DNA replication, is expressed through transcription to RNA and, in the case of messenger RNA (mRNA), subsequent translation into proteins (polypeptide chains) as shown in Figure 31.1. The process of protein synthesis is called translation because the "language" of the nucleotide sequence on the mRNA is translated into the language of an amino acid sequence. Translation requires a genetic code, through which the information contained in the nucleic acid sequence is expressed to produce a specific sequence of amino acids. Any alteration in the nucleic acid sequence may result in an incorrect amino acid being inserted into the polypeptide chain, potentially causing disease or even death of the organism. Newly made (nascent) proteins undergo a number of processes to achieve their functional form. They must fold properly, and misfolding can result in aggregation or degradation of the protein. Many proteins are covalently modified to activate them or alter their activities. Finally, proteins are targeted to their final intra- or extracellular destinations by signals present in the proteins themselves.

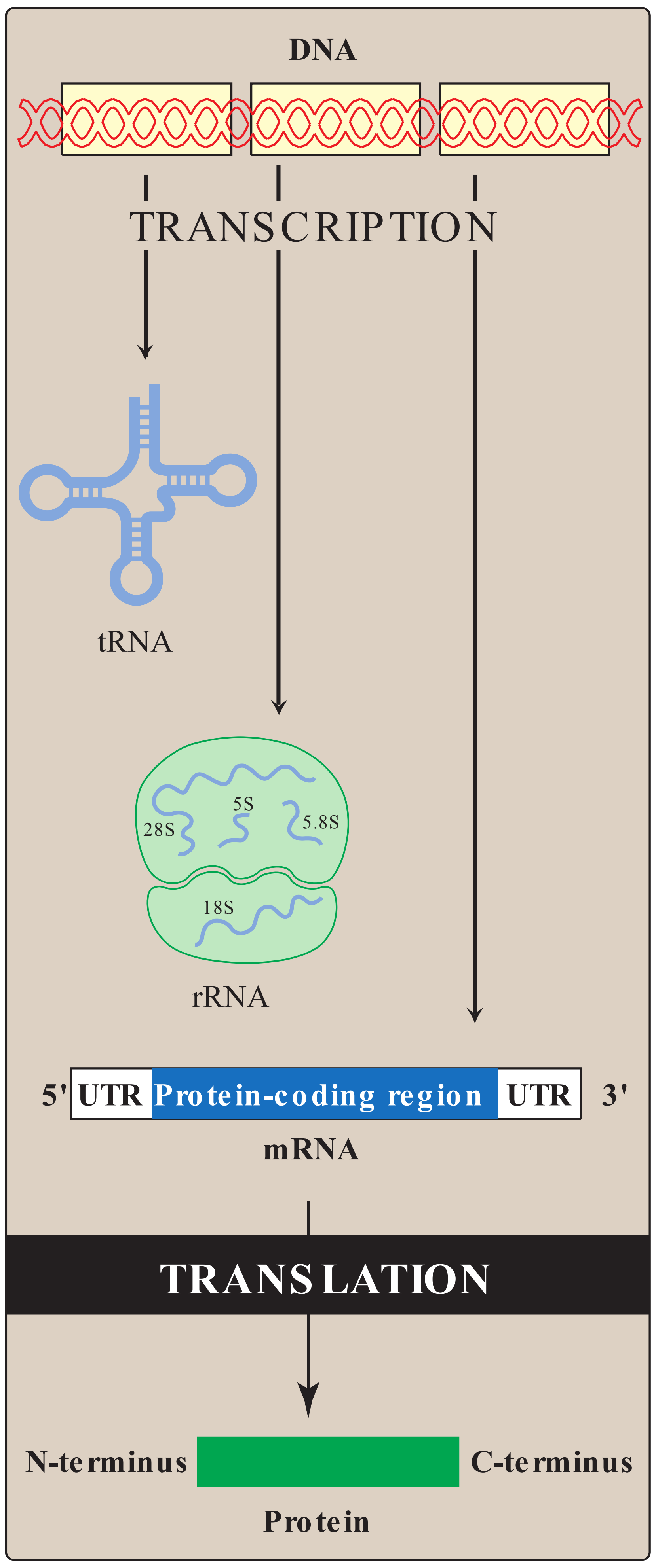


Figure 31.1
Protein synthesis or translation. tRNA = transfer RNA; rRNA = ribosomal RNA; mRNA = messenger RNA; UTR = untranslated region.

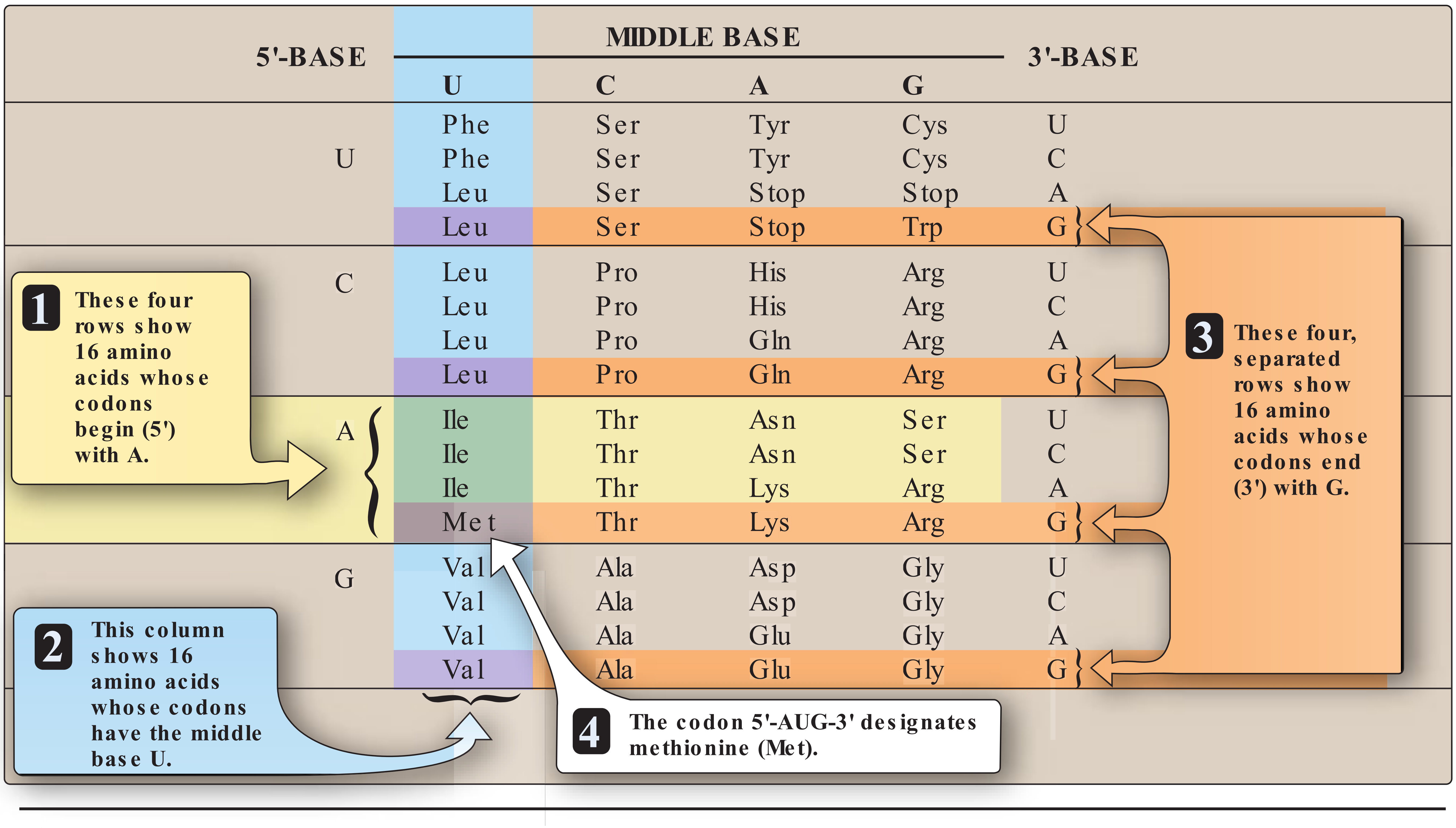
II. THE GENETIC CODE

The genetic code is a dictionary that identifies the correspondence between a sequence of nucleotide bases and a sequence of amino acids. Each individual "word" in the code is composed of three nucleotide bases. These genetic words are called codons.

A. Codons

Codons are presented in the mRNA language of adenine (A), guanine (G), cytosine (C), and uracil (U). Their nucleotide sequences are always written from the 5'-end to the 3'-end. The four nucleotide bases are used to produce the three-base codons. There are, therefore, 64 different combinations of bases, taken three at a time (a triplet code) as shown in the table in Figure 31.2.

1. **How to translate a codon:** This table (or "dictionary") can be used to translate any codon and, thus, to determine which amino acids are coded for by an mRNA sequence. For example, the codon 5'-AUG-3' codes for methionine ([Met] see Figure 31.2). [Note:



5'-BASE	MIDDLE BASE U	C	A	G	3'-BASE
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

1 These four rows show 16 amino acids whose codons begin (5') with A.

2 This column shows 16 amino acids whose codons have the middle base U.

3 These four, separated rows show 16 amino acids whose codons end (3') with G.

4 The codon 5'-AUG-3' designates methionine (Met).

Figure 31.2
Use of the genetic code table to translate the codon AUG. A = adenine; G = guanine; C = cytosine; U = uracil. The abbreviations for many common amino acids are shown as examples.

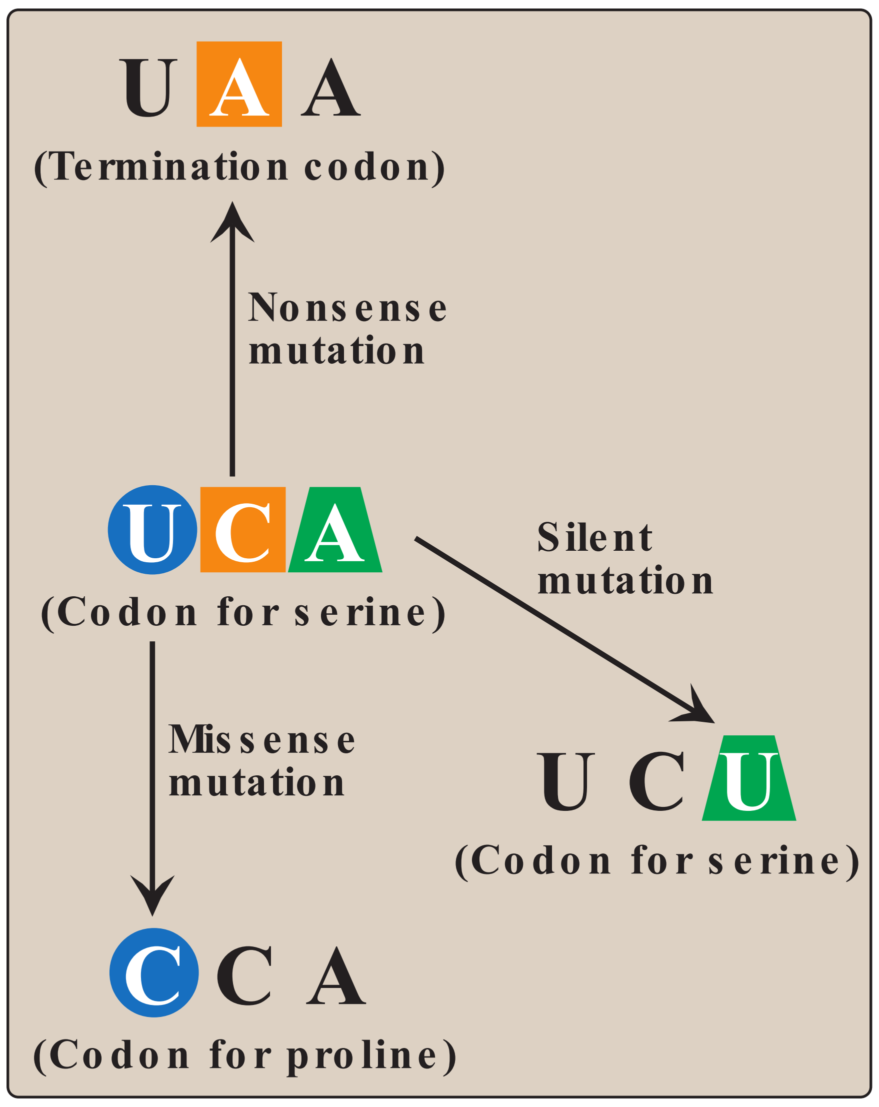


Figure 31.3
Possible effects of changing a single nucleotide base in the coding region of a messenger RNA chain. A = adenine; C = cytosine; U = uracil.

AUG is the initiation (start) codon for translation.] Sixty-one of the 64 codons code for the 20 common amino acids.

2. **Termination ("stop," or "nonsense") codons:** Three of the codons, UAA, UAG, and UGA, do not code for amino acids but, rather, are termination codons. When one of these codons appears in an mRNA sequence, synthesis of the polypeptide coded for by that mRNA stops.

B. Characteristics of the genetic code

Usage of the genetic code is remarkably consistent throughout all living organisms. It is assumed that once the standard genetic code evolved in primitive organisms, any mutation that altered its meaning would have caused the alteration of most, if not all, protein sequences, resulting in lethality. Characteristics of the genetic code include the following.

1. **Specificity:** The genetic code is specific (unambiguous), because a particular codon always codes for the same amino acid.

2. **Universality:** The genetic code is virtually universal insofar as its specificity has been conserved from very early stages of evolution, with only slight differences in the manner in which the code is translated. [Note: An exception occurs in mitochondria, in which a few codons have meanings different than those shown in Figure 31.2. For example, UGA codes for tryptophan (Trp).]

3. **Degeneracy:** The genetic code is degenerate (sometimes called redundant). Although each codon corresponds to a single amino acid, a given amino acid may have more than one triplet coding for it. For example, arginine (Arg) is specified by six different codons (see Figure 31.2). Only Met and Trp have just one coding triplet.

4. **Nonoverlapping and commaless:** The genetic code is nonoverlapping and commaless, meaning that the code is read from a fixed starting point as a continuous sequence of bases, taken three at a time without any punctuation between codons. For example, AGCUGGAUACAU is read as AGC UGG AUA CAU.

C. Consequences of altering the nucleotide sequence

Changing a single nucleotide base on the mRNA chain (a "point mutation") can lead to any one of three results (Figure 31.3).

1. **Silent mutation:** The codon containing the changed base may code for the same amino acid. For example, if the serine (Ser) codon UCA is given a different third base, U, to become UCU, it still codes for Ser. This is termed a "silent" mutation.

2. **Missense mutation:** The codon containing the changed base may code for a different amino acid. For example, if the Ser codon UCA is given a different first base, C, to become CCA, it will code for a different amino acid (in this case, proline [Pro]). The substitution of an incorrect amino acid is called a "missense" mutation.

3. **Nonsense mutation:** The codon containing the changed base may become a termination codon. For example, if the Ser codon UCA is given a different second base, A, to become UAA, the new codon causes termination of translation at that point and the production of a shortened (truncated) protein. The creation of a termination (stop) codon at an inappropriate place is called a "nonsense" mutation.

4. **Other mutations:** These can alter the amount or structure of the protein produced by translation.

   a. **Trinucleotide repeat expansion:** Occasionally, a sequence of three bases that is repeated in tandem will become amplified in number so that too many copies of the triplet occur. If this happens within the coding region of a gene, the protein will contain many extra copies of one amino acid. For example, amplification of the CAG codon leads to the insertion of many extra glutamine residues in the huntingtin protein, causing the neurodegenerative disorder Huntington disease (Figure 31.4). The additional glutamines result in changes in secondary structure that cause the accumulation of protein aggregates. If the trinucleotide repeat expansion occurs in an untranslated region (UTR) of a gene, the result can be a decrease in the amount of protein produced, as seen in fragile X syndrome and myotonic dystrophy. Over 20 triplet expansion diseases are known. [Note: In fragile X syndrome, the most common cause of intellectual disability, the expansion results in gene silencing through DNA hypermethylation (see p. 460).]

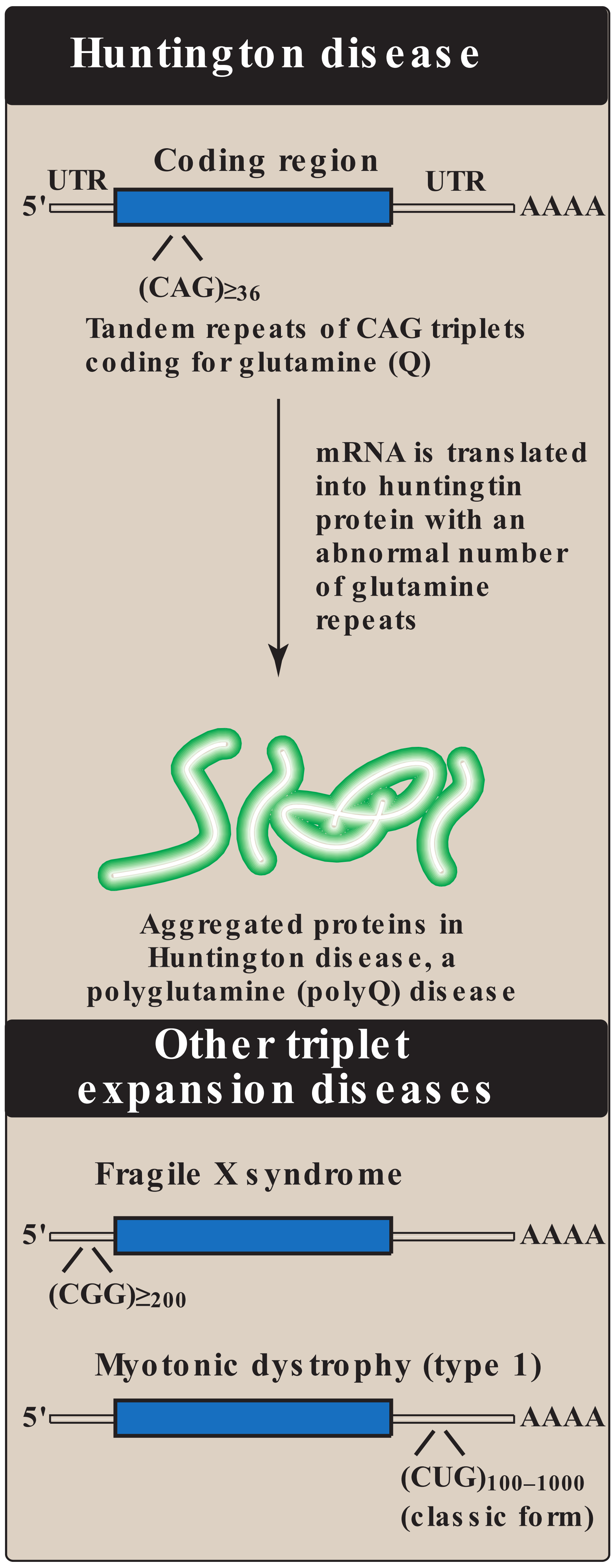

Huntington disease
UTR
Coding region
UTR
5'
AAAA
(CAG)≥36
Tandem repeats of CAG triplets coding for glutamine (Q)
mRNA is translated into huntingtin protein with an abnormal number of glutamine repeats
Aggregated proteins in Huntington disease, a polyglutamine (polyQ) disease
Other triplet expansion diseases
Fragile X syndrome
5'
AAAA
(CGG)≥200
Myotonic dystrophy (type 1)
5'
AAAA
(CUG)100–1000
(classic form)

Figure 31.4
Tandem triplet repeats in messenger RNA (mRNA) causing Huntington disease and other triplet expansion diseases. [Note: In unaffected individuals, the number of repeats in the huntingtin protein is fewer than 27, in fragile X mental retardation protein it is 5–44, and in myotonic dystrophy protein kinase it is 5–34.] UTR = untranslated region; A = adenine; C = cytosine; G= guanine; U = uracil; Q = single letter abbreviation for glutamine.

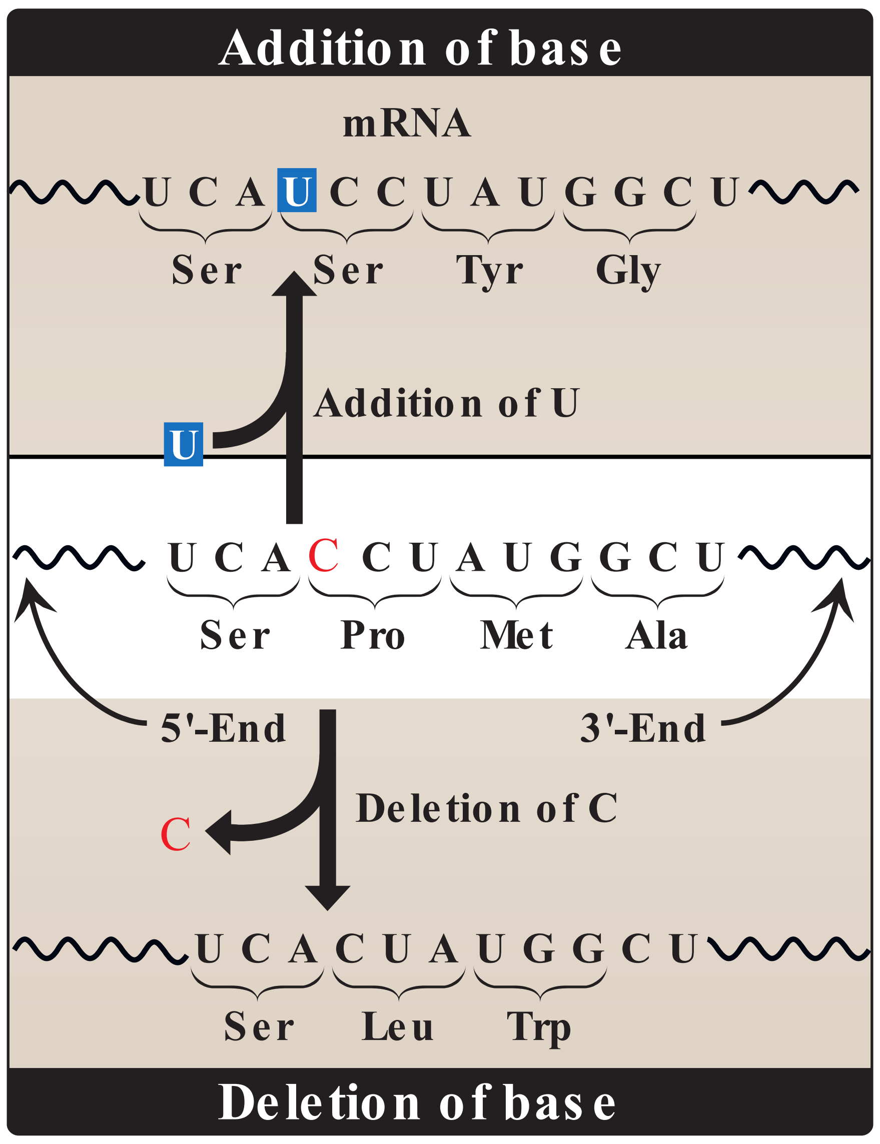


Figure 31.5
Frame-shift mutations as a result of addition or deletion of a base can cause an alteration in the reading frame of messenger RNA (mRNA). A = adenine; C = cytosine; G = guanine; U = uracil.

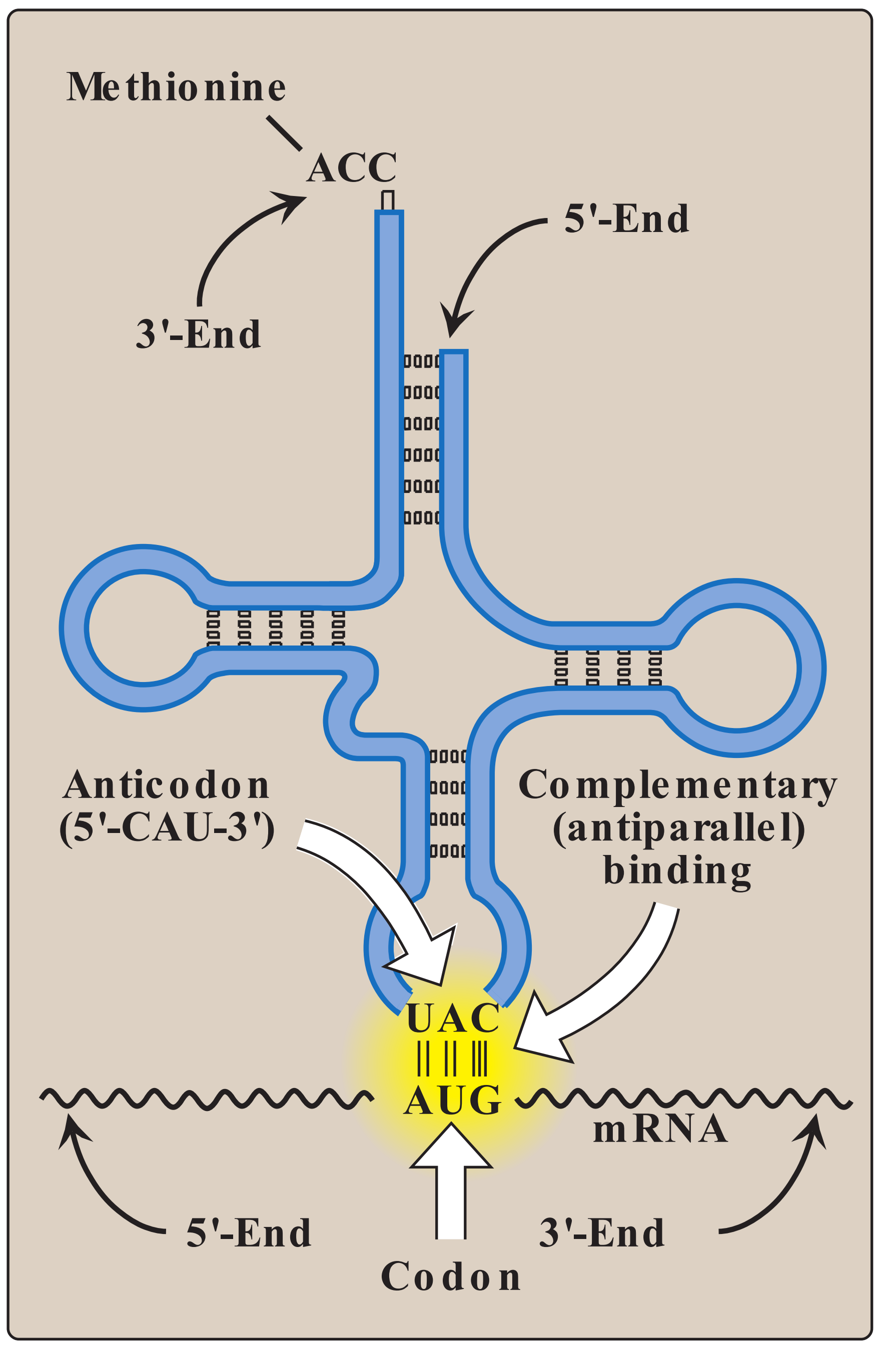


Figure 31.6
Complementary, antiparallel binding of the anticodon for methionyl-tRNA (CAU) to the messenger RNA (mRNA) codon for methionine (AUG), the initiation codon for translation.

b. Splice-site mutations: Mutations at splice sites (see p. 427) can alter the way in which introns are removed from pre-mRNA molecules, producing aberrant proteins. [Note: In myotonic dystrophy, a muscle disorder, gene silencing is the result of splicing alterations due to triplet expansion.]

c. Frame-shift mutations: If one or two nucleotides are either deleted from or added to the coding region of a mRNA, a frame-shift mutation occurs, altering the reading frame. This can result in a product with a radically different amino acid sequence or a truncated product due to the creation of a termination codon (Figure 31.5). If three nucleotides are added, a new amino acid is added to the peptide, or, if three are deleted, an amino acid is lost. Loss of three nucleotides maintains the reading frame but can result in serious pathology. For example, cystic fibrosis (CF), a chronic, progressive, inherited disease that primarily affects the pulmonary and digestive systems, is most commonly caused by deletion of three nucleotides from the coding region of a gene, resulting in the loss of phenylalanine at the 508th position (DF508) in the protein encoded by that gene. This DF508 mutation prevents normal folding of the protein, CF transmembrane conductance regulator (CFTR), leading to its destruction by the proteasome (see p. 247). CFTR normally functions as a chloride channel in epithelial cells, and its loss results in the production of thick, sticky secretions in the lungs and pancreas, leading to lung damage and digestive deficiencies (see p. 248). The incidence of CF is highest (1 in 3300) in those of Northern European origin. In over 70% of individuals with CF, the DF508 mutation is the cause of the disease.

III. COMPONENTS REQUIRED FOR TRANSLATION

A large number of components are required for the synthesis of a protein. These include all the amino acids that are found in the finished product, the mRNA to be translated, transfer RNA (tRNA) for each of the amino acids, functional ribosomes, energy sources, and enzymes as well as noncatalytic protein factors needed for the initiation, elongation, and termination steps of polypeptide chain synthesis.

A. Amino acids

All the amino acids that eventually appear in the finished protein must be present at the time of protein synthesis. If one amino acid is missing, translation stops at the codon specifying that amino acid. [Note: This demonstrates the importance of having all the essential amino acids (see p. 262) in sufficient quantities in the diet to ensure continued protein synthesis.]

B. Transfer RNA

At least one specific type of tRNA is required for each amino acid. In humans, there are at least 50 species of tRNA, whereas bacteria contain at least 30 species. Because there are only 20 different amino acids commonly carried by tRNA, some amino acids have more than one specific tRNA molecule. This is particularly true of those amino acids that are coded for by several codons.

1. **Amino acid attachment site:** Each tRNA molecule has an attachment site for a specific (cognate) amino acid at its 3'-end (Figure 31.6). The carboxyl group of the amino acid is in an ester linkage with the 3'-hydroxyl of the ribose portion of the adenine (A) nucleotide in the —CCA sequence at the 3'-end of the tRNA. [Note: When a tRNA has a covalently attached amino acid, it is said to be charged, and when it does not, it is said to be uncharged. The amino acid attached to the tRNA molecule is said to be activated.]

2. **Anticodon:** Each tRNA molecule also contains a three-base nucleotide sequence, the anticodon, that pairs with a specific codon on the mRNA (see Figure 31.6). This codon specifies the insertion into the growing peptide chain of the amino acid carried by that tRNA.

C. Aminoacyl-tRNA synthetases

This family of enzymes is required for attachment of amino acids to their corresponding tRNAs. Each member of this family recognizes a specific amino acid and all the tRNAs that correspond to that amino acid (isoaccepting tRNAs, up to five per amino acid). Aminoacyl-tRNA synthetases catalyze a two-step reaction that results in the covalent attachment of the carboxyl group of an amino acid to the 3'-end of its corresponding tRNA. The overall reaction requires adenosine triphosphate (ATP), which is cleaved to adenosine monophosphate (AMP) and inorganic pyrophosphate (PP$_i$) as shown in Figure 31.7. The extreme specificity of the synthetases in recognizing both the amino acid and its cognate tRNA contributes to the high fidelity of translation of the genetic message. In addition to their synthetic activity, the aminoacyl-tRNA synthetases have a "proofreading" or "editing" activity that can remove an incorrect amino acid from the enzyme or the tRNA molecule.

D. Messenger RNA

The specific mRNA required as a template for the synthesis of the desired polypeptide chain must be present. [Note: In eukaryotes, mRNA is circularized for use in translation.]

E. Functionally competent ribosomes

Ribosomes are large complexes of protein and ribosomal RNA ([rRNA], Figure 31.8), in which rRNA predominates. They consist of two subunits (one large and one small) whose relative sizes are given in terms of their sedimentation coefficients, or S (Svedberg) values. [Note: Because the S values are determined both by shape as well as molecular mass, their numeric values are not strictly additive. For example, the prokaryotic 50S and 30S ribosomal subunits together form a 70S ribosome. The eukaryotic 60S and 40S subunits form an 80S ribosome.] Prokaryotic and eukaryotic ribosomes are similar in structure, and serve the same function, namely, as

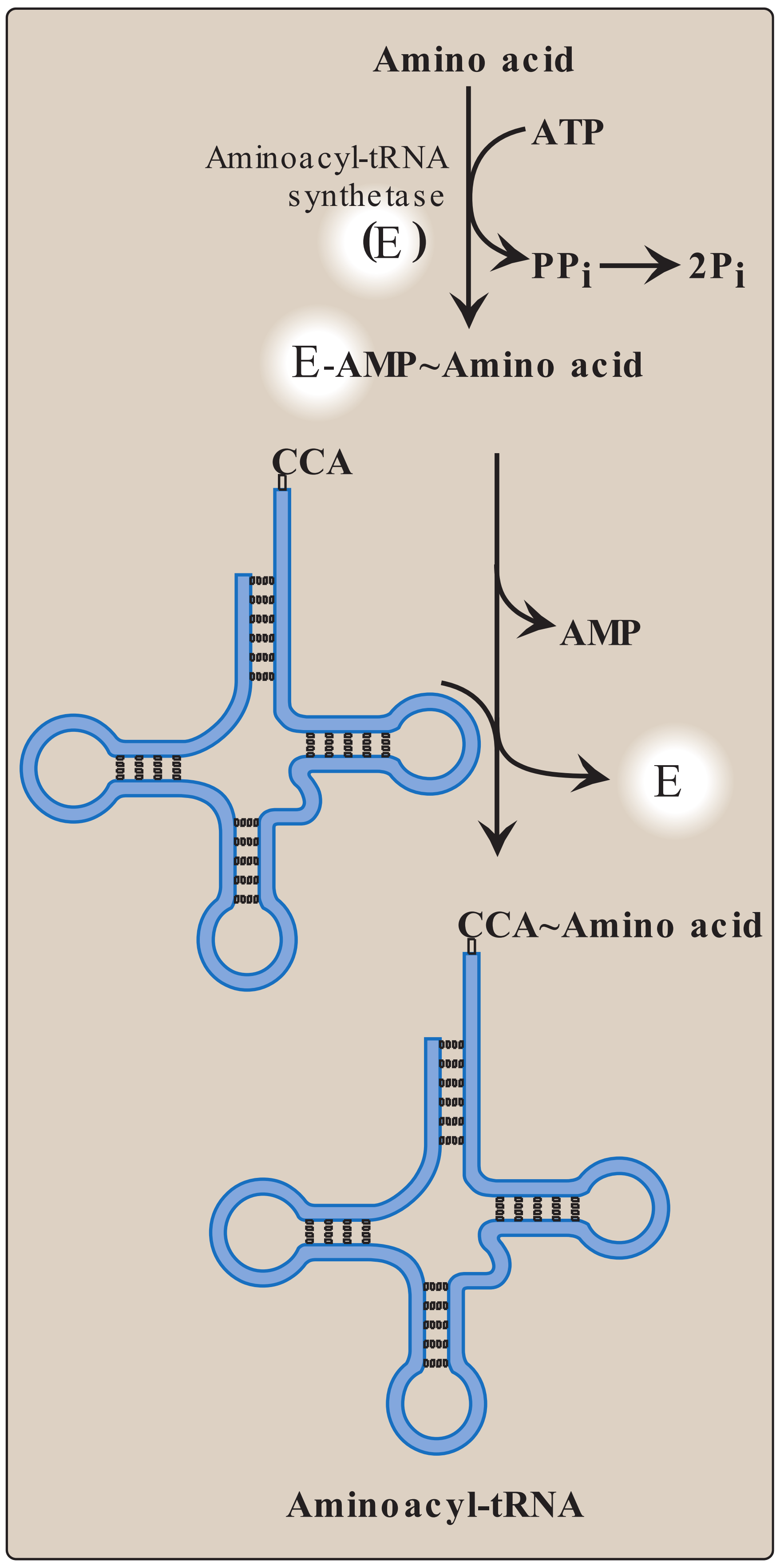


Figure 31.7
Attachment of a specific amino acid to its corresponding tRNA by aminoacyl-tRNA synthetase. PP$_i$ = pyrophosphate; P$_i$ = inorganic phosphate; A = adenine; C = cytosine; ATP = adenosine triphosphate; AMP = adenosine monophosphate.

the macromolecular complexes in which the synthesis of proteins occurs.

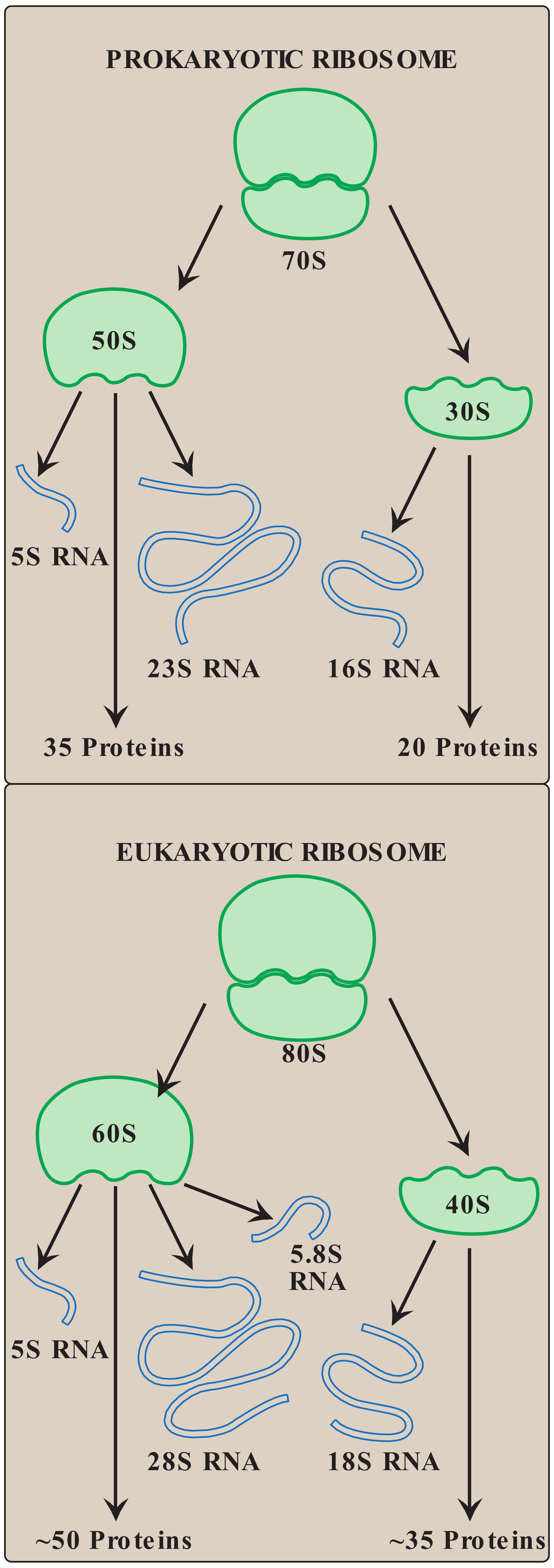


Figure 31.8
Ribosomal composition. [Note: The number of proteins in the eukaryotic ribosomal subunits varies somewhat from species to species.]
S = Svedberg unit.

> The small ribosomal subunit binds mRNA and is responsible for the accuracy of translation by ensuring correct base-pairing between the codon in the mRNA and the anticodon in the tRNA. The large ribosomal subunit catalyzes formation of the peptide bonds that link amino acid residues in a protein.

1. **Ribosomal RNA:** As discussed on p. 418, prokaryotic ribosomes contain three size species of rRNA, whereas eukaryotic ribosomes contain four (see Figure 31.8). The rRNAs are generated from a single pre-rRNA by the action of ribonucleases, and some bases and riboses are modified.

2. **Ribosomal proteins:** Ribosomal proteins are present in greater numbers in eukaryotic ribosomes than in prokaryotic ribosomes. These proteins play a variety of roles in the structure and function of the ribosome and its interactions with other components of the translation system.

3. **A, P, and E sites on the ribosome:** The ribosome has three binding sites for tRNA molecules: the A, P, and E sites, each of which extends over both subunits. Together, they cover three neighboring codons. During translation, the A site binds an incoming aminoacyl-tRNA as directed by the codon currently occupying this site. This codon specifies the next amino acid to be added to the growing peptide chain. The P-site codon is occupied by peptidyl-tRNA. This tRNA carries the chain of amino acids that has already been synthesized. The E site is occupied by the empty tRNA as it is about to exit the ribosome. (See Figure 31.13 for an illustration of the role of the A, P, and E sites in translation.)

4. **Cellular location of ribosomes:** In eukaryotic cells, the ribosomes are either “free” in the cytosol or are in close association with the endoplasmic reticulum (which is then known as the “rough” endoplasmic reticulum, or RER). The RER-associated ribosomes are responsible for synthesizing proteins that are to be exported from the cell as well as those that are destined to become incorporated into plasma, endoplasmic reticulum, or Golgi membranes or imported into lysosomes (see p. 169 for an overview of the latter process). Cytosolic ribosomes synthesize proteins required in the cytosol itself or destined for the nucleus, mitochondria or peroxisomes. [Note: Mitochondria contain their own set of ribosomes and their own unique, circular DNA. Most mitochondrial proteins, however, are encoded by nuclear DNA, synthesized in the cytosol, and posttranslationally targeted to mitochondria.]

F. Protein factors

Initiation, elongation, and termination (or release) factors are required for peptide synthesis. Some of these protein factors perform a catalytic function, whereas others appear to stabilize the synthetic machinery. [Note: A number of the factors are monomeric G proteins, and thus are active when bound to guanosine triphosphate (GTP) and inactive when bound to guanosine diphosphate (GDP) (see p. 95 for a discussion of the heterotrimeric G proteins).]

G. ATP and GTP are required as sources of energy

Cleavage of four high-energy bonds is required for the addition of one amino acid to the growing polypeptide chain: two from ATP in the aminoacyl-tRNA synthetase reaction—one in the removal of PP_i, and one in the subsequent hydrolysis of the PP_i to inorganic phosphate by pyrophosphatase —and two from GTP—one for binding the aminoacyl-tRNA to the A site and one for the translocation step (see Figure 31.13, p. 440). [Note: Additional ATP and GTP molecules are required for initiation in eukaryotes, whereas an additional GTP molecule is required for termination in both eukaryotes and prokaryotes.]

IV. CODON RECOGNITION BY TRANSFER RNA

Correct pairing of the codon in the mRNA with the anticodon of the tRNA is essential for accurate translation (see Figure 31.6). Some tRNAs (isoaccepting tRNAs) recognize more than one codon for a given amino acid.

A. Antiparallel binding between codon and anticodon

Binding of the tRNA anticodon to the mRNA codon follows the rules of complementary and antiparallel binding, that is, the mRNA codon is "read" 5'→3' by an anticodon pairing in the "flipped" (3'→5') orientation (Figure 31.9). [Note: Nucleotide sequences are always assumed to be written in the 5' to 3' direction unless otherwise noted. Two nucleotide sequences orient in an antiparallel manner.]

B. Wobble hypothesis

The mechanism by which tRNAs can recognize more than one codon for a specific amino acid is described by the "wobble" hypothesis, which states that codon–anticodon pairing follows the traditional Watson-Crick rules (C pairs with G and A pairs with U) for the first two bases of the codon but can be less stringent for the last base. The base at the 5'-end of the anticodon (the "first" base of the anticodon) is not as spatially defined as the other two bases. Movement of that first base allows nontraditional base-pairing with the 3'-base of the codon (the "last" base of the codon). This movement is called wobble and allows a single tRNA to recognize more than one codon. Examples of these flexible pairings are shown in Figure 31.9. The result of wobble is that there need not be 61 tRNA species to read the 61 codons that code for amino acids.

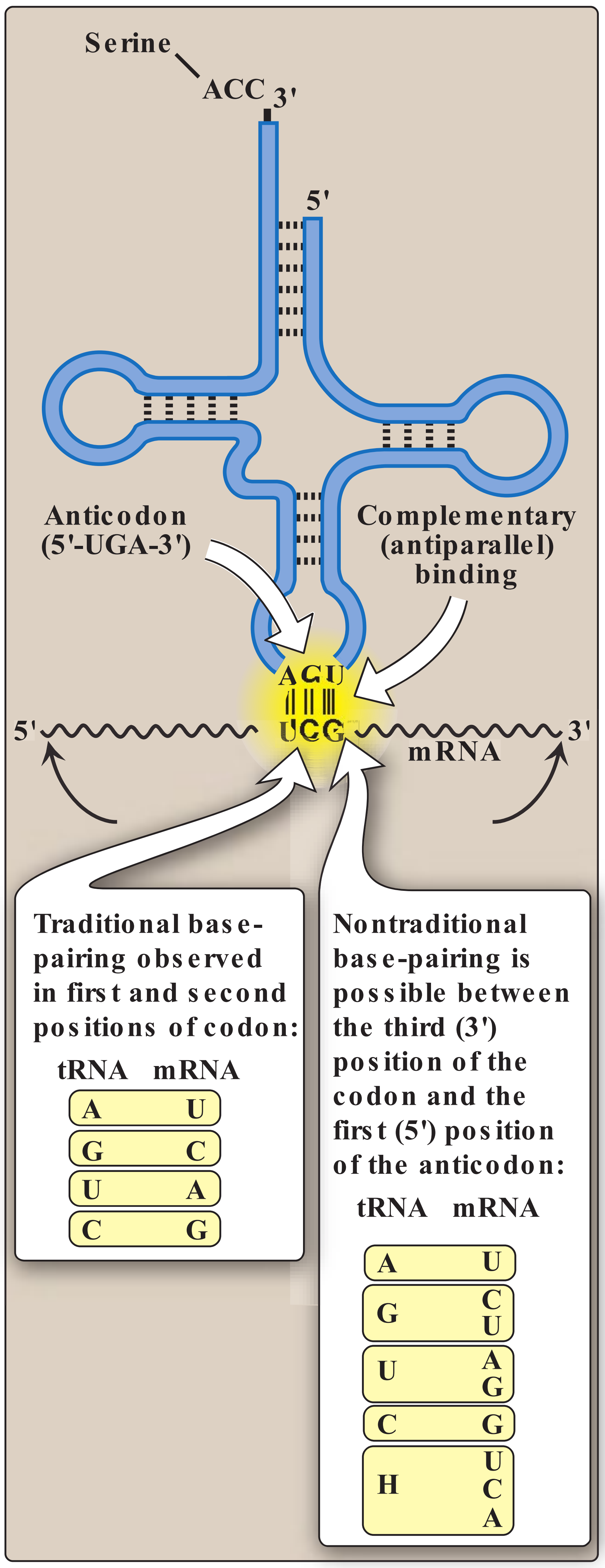

Serine
ACC 3'
5'
Anticodon (5'-UGA-3')
Complementary (antiparallel) binding
ACU
UCG
5'
3'
mRNA
Traditional base-pairing observed in first and second positions of codon:
tRNA mRNA
A U
G C
U A
C G
Nontraditional base-pairing is possible between the third (3') position of the codon and the first (5') position of the anticodon:
tRNA mRNA
A U
G C U
U A G
C G
H U C A

Figure 31.9
Wobble: Nontraditional base-pairing between the 5'-nucleotide (first nucleotide) of the anticodon with the 3'-nucleotide (last nucleotide) of the codon. Hypoxanthine (H) is the product of adenine deamination and the base in the nucleotide, inosine monophosphate (IMP). A = adenine; G = guanine; C = cytosine; U = uracil; tRNA = transfer RNA; mRNA = messenger RNA.

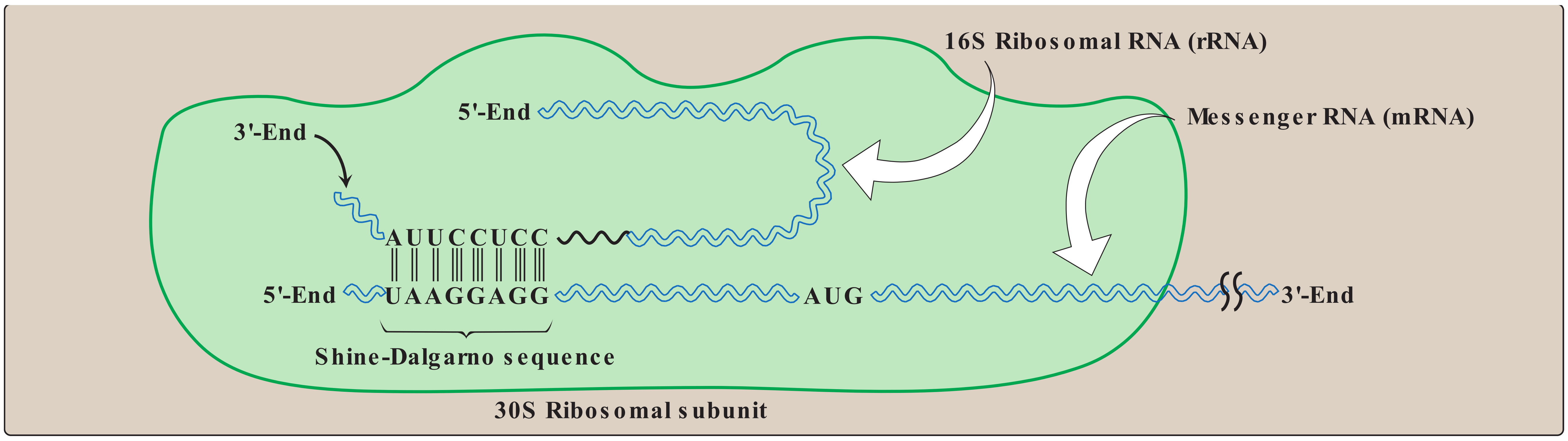

16S Ribosomal RNA (rRNA)
Messenger RNA (mRNA)
3'-End
5'-End
AUUCCUCC
5'-End
UAAGGAGG
AUG
3'-End
Shine-Dalgarno sequence
30S Ribosomal subunit

Figure 31.10
Complementary binding between prokaryotic mRNA Shine-Dalgarno sequence and 16S rRNA. S = Svedberg unit.

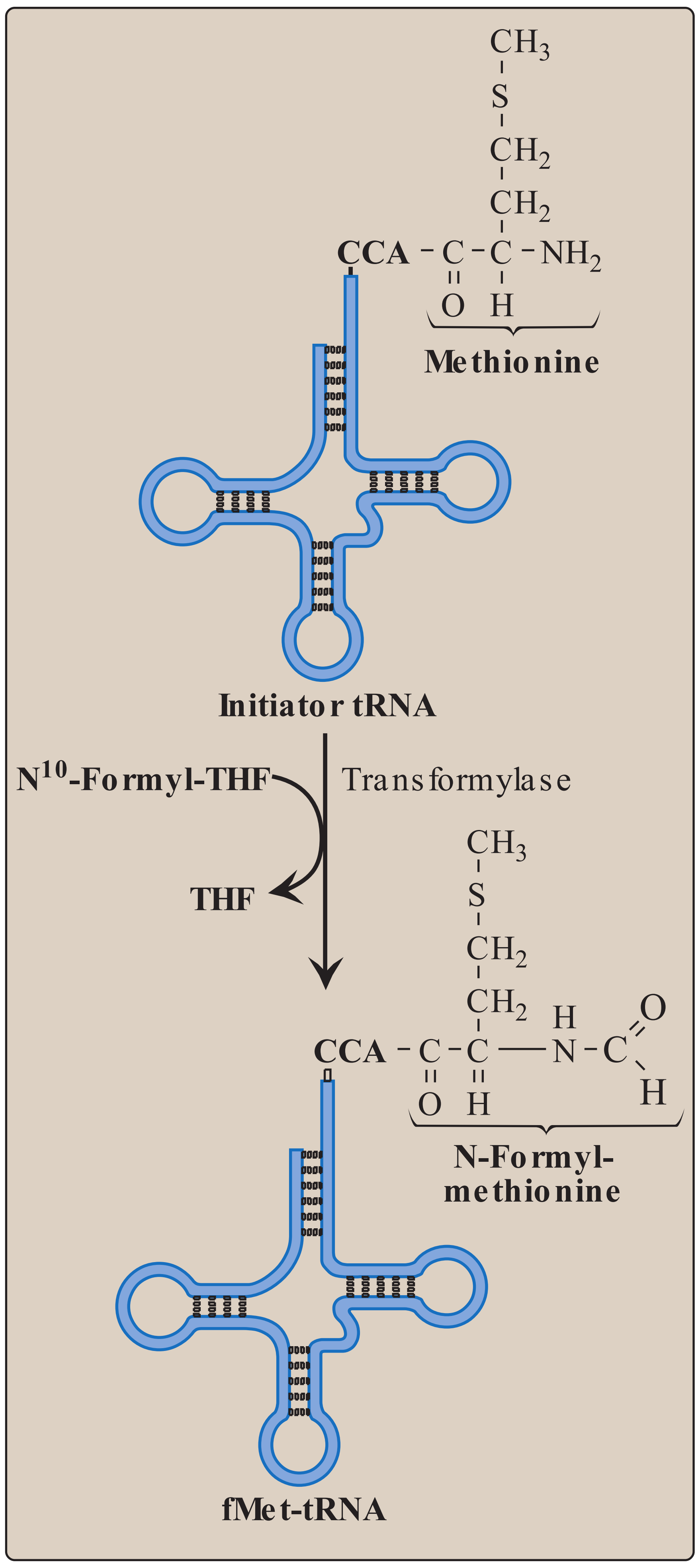

CH_3
S
CH_2
CH_2
CCA
C
C
NH_2
O
H
Methionine
Initiator tRNA
N^{10}-Formyl-THF
Transformylase
THF
CH_3
S
CH_2
CH_2
H
O
CCA
C
C
N
C
O
H
H
N-Formyl-methionine
fMet-tRNA

Figure 31.11
Generation of the initiator N-formyl-methionyl-tRNA (fMet-tRNA).
THF = tetrahydrofolate; C = cytosine; A = adenine.

V. STEPS IN PROTEIN SYNTHESIS

The process of protein synthesis translates the 3-letter alphabet of nucleotide sequences on mRNA into the 20-letter alphabet of amino acids that constitute proteins. The mRNA is translated from its 5'-end to its 3'-end, producing a protein synthesized from its amino (N)-terminal end to its carboxyl (C)-terminal end. Prokaryotic mRNAs often have several coding regions (that is, they are polycistronic; see p. 418). Each coding region has its own initiation and termination codon and produces a separate species of polypeptide. In contrast, each eukaryotic mRNA has only one coding region (that is, it is monocistronic). The process of translation is divided into three separate steps: initiation, elongation, and termination. Eukaryotic protein synthesis resembles that of prokaryotes in most aspects. Individual differences are noted in the text.

> One important difference is that translation and transcription are temporally linked in prokaryotes, with translation starting before transcription is completed as a consequence of the lack of a nuclear membrane in prokaryotes.

A. Initiation

Initiation of protein synthesis involves the assembly of the components of the translation system before peptide bond formation occurs. These components include the two ribosomal subunits, the mRNA to be translated, the aminoacyl-tRNA specified by the first codon in the message, GTP (which provides energy for the process), and initiation factors that facilitate the assembly of this initiation complex (see Figure 31.13). [Note: In prokaryotes, three initiation factors are known (IF-1, IF-2, and IF-3), whereas in eukaryotes, there are many (designated eIF to indicate eukaryotic origin). Eukaryotes also require ATP for initiation.] The following are two mechanisms by which the ribosome recognizes the nucleotide sequence (AUG) that initiates translation.

1. **Shine-Dalgarno sequence:** In Escherichia coli (E. coli), a purine-rich sequence of nucleotide bases, known as the Shine-Dalgarno

(SD) sequence, is located six to ten bases upstream of the initiating AUG codon on the mRNA molecule (that is, near its 5'-end). The 16S rRNA component of the small (30S) ribosomal subunit has a nucleotide sequence near its 3'-end that is complementary to all or part of the SD sequence. Therefore, the 5'-end of the mRNA and the 3'-end of the 16S rRNA can form complementary base pairs, facilitating the positioning of the small ribosomal subunit on the mRNA in close proximity to the initiating AUG codon (Figure 31.10).

2. **5' Cap:** Eukaryotic mRNAs do not have SD sequences. In eukaryotes, the small (40S) ribosomal subunit (aided by members of the eIF-4 family of proteins) binds close to the cap structure at the 5'-end of the mRNA and moves down the mRNA until it encounters the initiator AUG. This "scanning" process requires ATP. [Note: Interactions between the cap-binding eIF-4 proteins and the poly-A tail-binding proteins on eukaryotic mRNA mediate circularization of the mRNA and likely prevent the use of incompletely processed mRNA in translation.]

3. **Initiation codon:** The initiating AUG is recognized by a special initiator tRNA. Recognition is facilitated by IF-2-GTP in prokaryotes and eIF-2-GTP (plus additional eIFs) in eukaryotes. The charged initiator tRNA enters the P site on the small subunit. The initiator tRNA is the only tRNA recognized by (e)IF-2 and the only tRNA to go directly to the P site. In bacteria and in mitochondria, the initiator tRNA carries an N-formylated methionine (fMet, Figure 31.11). After Met is attached to the initiator tRNA, the formyl group is added by the enzyme transformylase, which uses N^{10}-formyl tetrahydrofolate (see p. 267) as the carbon donor. In eukaryotes, the initiator tRNA carries a Met that is not formylated. In both prokaryotic and eukaryotic cells, this N-terminal Met is usually removed before translation is completed. The large ribosomal subunit then joins the complex, and a functional ribosome is formed with the charged initiating tRNA in the P site. The A site is empty. [Note: Specific (e)IFs function as anti-association factors and prevent premature addition of the large subunit.] The GTP on (e)IF-2 gets hydrolyzed to GDP. A guanine nucleotide exchange factor facilitates the reactivation of (e)IF-2-GDP through replacement of GDP by GTP.

B. Elongation

Elongation of the polypeptide chain involves the addition of amino acids to the carboxyl end of the growing chain. During elongation, the ribosome moves from the 5'-end to the 3'-end of the mRNA that is being translated. Delivery of the aminoacyl-tRNA whose codon appears next on the mRNA template in the ribosomal A site (a process known as decoding) is facilitated in E. coli by elongation factors EF-Tu-GTP and EF-Ts and requires GTP hydrolysis. [Note: In eukaryotes, comparable elongation factors are EF-1a-GTP and EF-1bg. Both EF-Ts and EF-1bg function in guanine nucleotide exchange.] The formation of the peptide bond is catalyzed by peptidyltransferase, an activity intrinsic to the 23S rRNA found in the large (50S) ribosomal subunit (Figure 31.12). [Note: Because this rRNA catalyzes the reaction, it is referred to as a ribozyme.] After the peptide

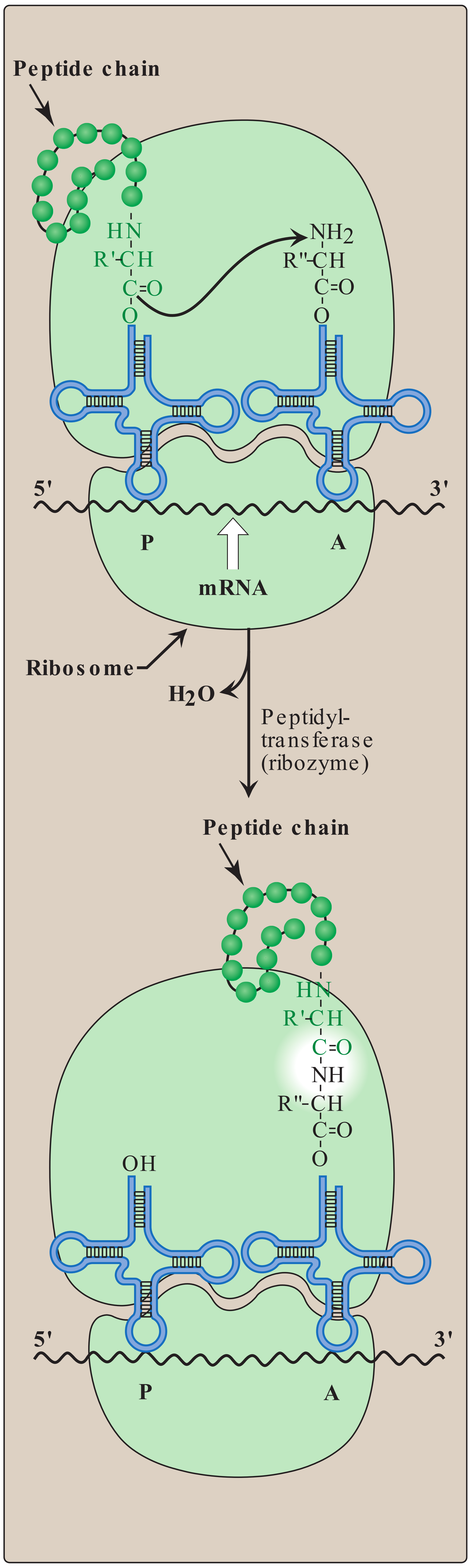
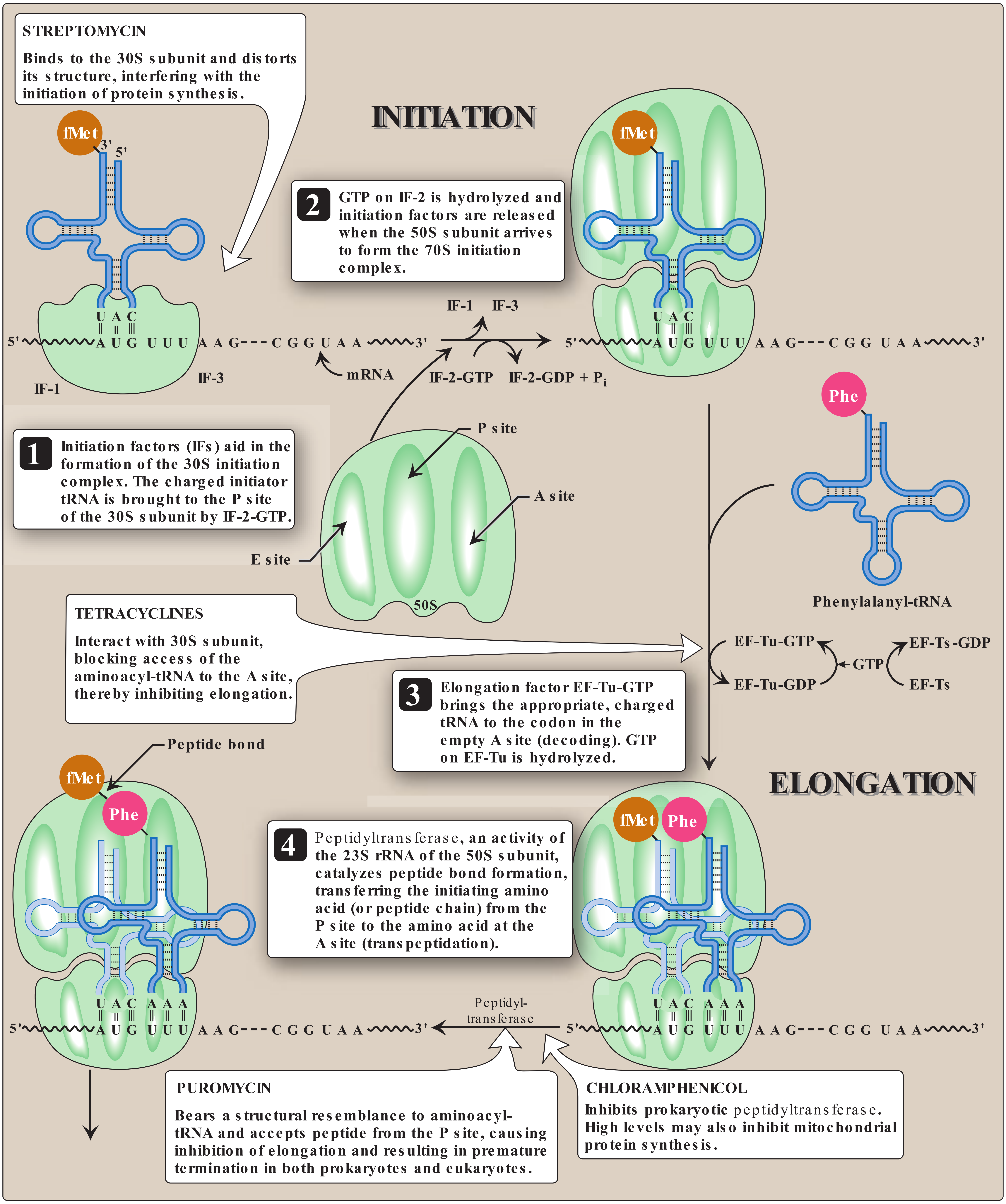


Figure 31.12
Formation of a peptide bond. Peptide bond formation involves transfer of the peptide on the transfer RNA (tRNA) in the P site to the amino acid on the tRNA in the A site (transpeptidation). mRNA = messenger RNA.


INITIATION
fMet
3'
5'
U A C
5' A U G U U U A A G - - - C G G U A A 3'
IF-1
IF-3
mRNA
IF-1 IF-3
IF-2-GTP
IF-2-GDP + P_i
P site
A site
E site
50S
Phe
Phenylalanyl-tRNA
EF-Tu-GTP
GTP
EF-Ts-GDP
EF-Tu-GDP
EF-Ts
ELONGATION
Peptide bond
U A C A A A
Peptidyl-transferase

STREPTOMYCIN

Binds to the 30S subunit and distorts its structure, interfering with the initiation of protein synthesis.

2 GTP on IF-2 is hydrolyzed and initiation factors are released when the 50S subunit arrives to form the 70S initiation complex.

1 Initiation factors (IFs) aid in the formation of the 30S initiation complex. The charged initiator tRNA is brought to the P site of the 30S subunit by IF-2-GTP.

TETRACYCLINES

Interact with 30S subunit, blocking access of the aminoacyl-tRNA to the A site, thereby inhibiting elongation.

3 Elongation factor EF-Tu-GTP brings the appropriate, charged tRNA to the codon in the empty A site (decoding). GTP on EF-Tu is hydrolyzed.

4 Peptidyltransferase, an activity of the 23S rRNA of the 50S subunit, catalyzes peptide bond formation, transferring the initiating amino acid (or peptide chain) from the P site to the amino acid at the A site (transpeptidation).

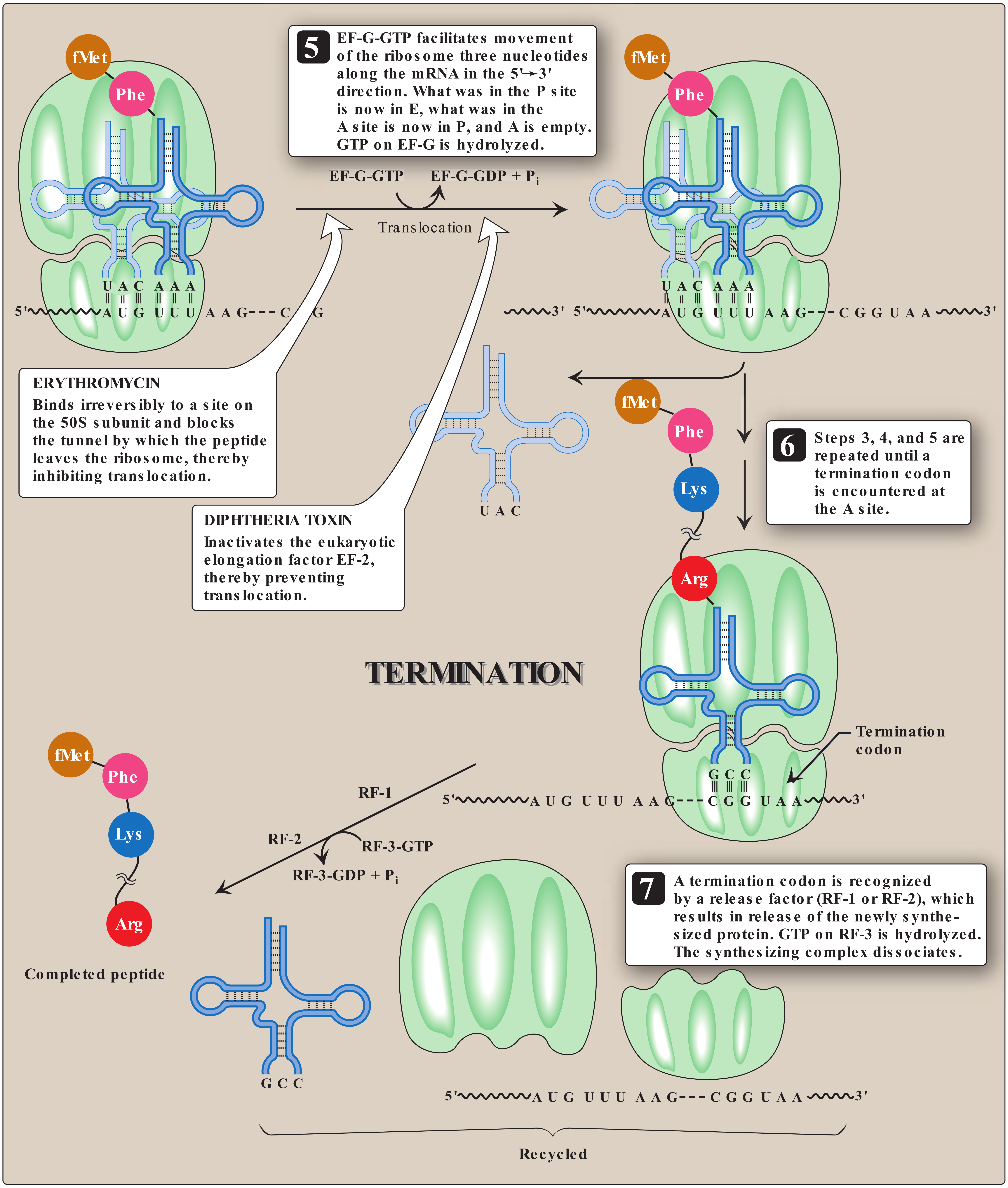
PUROMYCIN

Bears a structural resemblance to aminoacyl-tRNA and accepts peptide from the P site, causing inhibition of elongation and resulting in premature termination in both prokaryotes and eukaryotes.

CHLORAMPHENICOL

Inhibits prokaryotic peptidyltransferase. High levels may also inhibit mitochondrial protein synthesis.

Figure 31.13 (continued on the next page)
Steps in prokaryotic protein synthesis (translation), and their inhibition by antibiotics. [Note: EF-Ts is a guanine nucleotide exchange factor. It facilitates the removal of GDP, allowing its replacement by GTP. The eukaryotic equivalent is EF-1βγ.] fMet = formylated methionine; S = Svedberg unit; GTP = guanine nucleoside triphosphate; Phe = phenylalanine.



5 EF-G-GTP facilitates movement of the ribosome three nucleotides along the mRNA in the 5'→3' direction. What was in the P site is now in E, what was in the A site is now in P, and A is empty. GTP on EF-G is hydrolyzed.

ERYTHROMYCIN
Binds irreversibly to a site on the 50S subunit and blocks the tunnel by which the peptide leaves the ribosome, thereby inhibiting translocation.

DIPHTHERIA TOXIN
Inactivates the eukaryotic elongation factor EF-2, thereby preventing translocation.

6 Steps 3, 4, and 5 are repeated until a termination codon is encountered at the A site.

7 A termination codon is recognized by a release factor (RF-1 or RF-2), which results in release of the newly synthesized protein. GTP on RF-3 is hydrolyzed. The synthesizing complex dissociates.

fMet
Phe
U A C A A A
5' A U G U U U A A G - - - C G
EF-G-GTP
EF-G-GDP + P_i
Translocation
3'
fMet
Phe
U A C A A A
5' A U G U U U A A G - - - C G G U A A 3'
U A C
fMet
Phe
Lys
Arg
TERMINATION
G C C
5' A U G U U U A A G - - - C G G U A A 3'
Termination codon
fMet
Phe
Lys
Arg
Completed peptide
RF-1
RF-2
RF-3-GTP
RF-3-GDP + P_i
G C C
5' A U G U U U A A G - - - C G G U A A 3'
Recycled

Figure 31.13 (Continued from the previous page)
[Note: Ricin, a toxin from castor beans, removes an A from the 28 S rRNA in the large subunit of eukaryotic ribosomes, thereby inhibiting their function.] Lys = lysine; Arg = arginine.

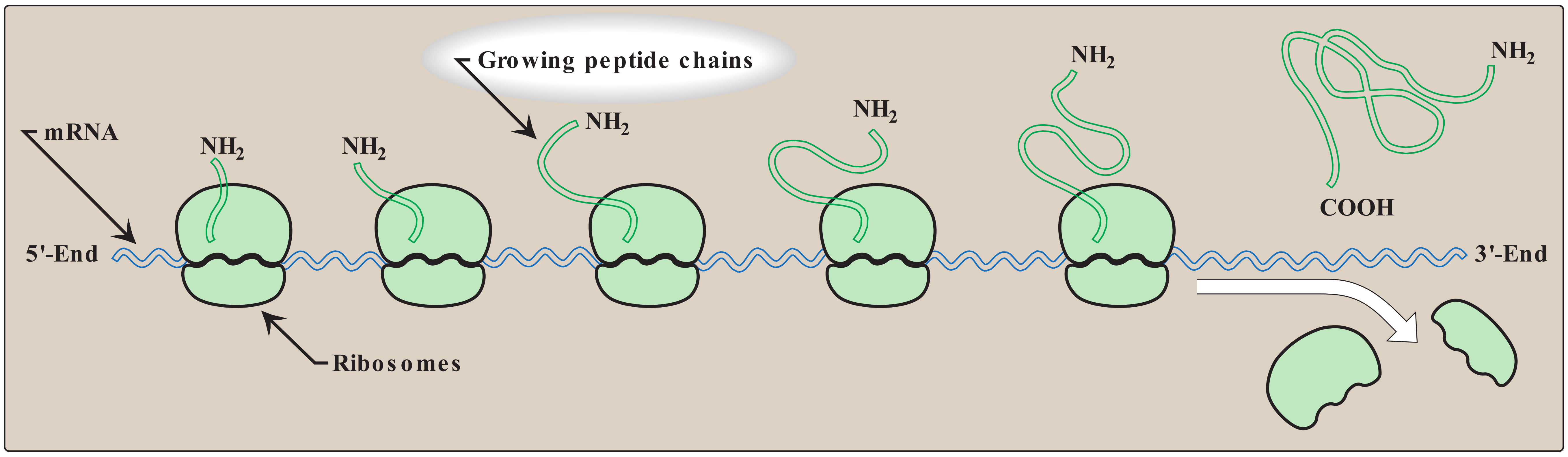


Figure 31.14
A polyribosome consists of several ribosomes simultaneously translating one messenger RNA (mRNA). [Note: Eukaryotic mRNA is circularized for translation.]

bond has been formed, what was attached to the tRNA at the P site is now linked to the amino acid on the tRNA at the A site. The ribosome then advances three nucleotides toward the 3'-end of the mRNA. This process is known as translocation and, in prokaryotes, requires the participation of EF-G-GTP (eukaryotic cells use EF-2-GTP) and GTP hydrolysis. Translocation causes movement of the uncharged tRNA from the P to the E site for release and movement of the peptidyl-tRNA from the A to the P site. The process is repeated until a termination codon is encountered.

Cell	Factor	Function
	Initiation	
P E	IF-2-GTP eIF-2-GTP	Bring charged initiating tRNA to P site
P E	IF-3 eIF-3	Prevent association of subunits
	Elongation	
P E	EF-Tu-GTP EF1α-GTP	Bring all other charged tRNAs to A site
P E	EF-Ts EF-1$\beta\gamma$	Guanine nucleotide exchange factor
P E	EF-G-GTP EF-2-GTP	Translocation
	Termination	
P E	RF-1, 2 eRF	Recognize "stop" codons
P E	RF-3-GTP eRF-3-GTP	Release of other RFs

Figure 31.15
Protein factors in the three stages of translation. P = prokaryotes; E = eukaryotes; tRNA = transfer RNA; IF = initiation factor; EF = elongation factor; RF = release factor.

C. Termination

Termination occurs when one of the three termination codons moves into the A site. These codons are recognized in E. coli by release factors: RF-1, which recognizes the termination codons UAA and UAG, and RF-2, which recognizes UGA and UAA. The binding of these release factors results in hydrolysis of the bond linking the peptide to the tRNA at the P site, causing the nascent protein to be released from the ribosome. A third release factor, RF-3-GTP then causes the release of RF-1 or RF-2 as GTP is hydrolyzed (see Figure 31.13). [Note: Eukaryotes have a single release factor, eRF, which recognizes all three termination codons. A second factor, eRF-3, functions like the prokaryotic RF-3. See Figure 31.15 for a summary of the factors used in translation.] The steps in prokaryotic protein synthesis are summarized in Figure 31.13. The newly synthesized polypeptide may undergo further modification as described below, and the ribosomal subunits, mRNA, tRNA, and protein factors can be recycled and used to synthesize another polypeptide. [Note: In prokaryotes, ribosome recycling factors mediate separation of the subunits.] Some antibiotic inhibitors of protein synthesis are illustrated in Figure 31.13, as is diphtheria toxin.

D. Polysomes

Translation begins at the 5'-end of the mRNA, with the ribosome proceeding along the RNA molecule. Because of the length of most mRNAs, more than one ribosome at a time can translate a message (Figure 31.14). Such a complex of one mRNA and a number of ribosomes is called a polysome or polyribosome.

E. Regulation of translation

Gene expression is most commonly regulated at the transcriptional level, but translation may also be regulated. An important mechanism by which this is achieved in eukaryotes is by covalent modification of eIF-2: phosphorylated eIF-2 is inactive. In both eukaryotes and prokaryotes, regulation can also be achieved through proteins that bind mRNA and inhibit its use by blocking translation or extend its use by protecting it from degradation. For a more detailed discussion of the regulation of translation, see p. 454.

F. Protein targeting

Although most protein synthesis in eukaryotes is initiated in the cytoplasm, many proteins perform their functions within subcellular organelles or outside of the cell. Such proteins usually contain amino acid sequences that direct the proteins to their final locations. For example, proteins destined for secretion from the cell are targeted during their synthesis (cotranslational targeting) to the RER (see p. 436) by the presence of an N-terminal hydrophobic signal sequence. [Note: The sequence is recognized and bound by the signal recognition particle, which facilitates transport to the RER.] Proteins targeted after synthesis (posttranslational) include nuclear proteins that contain an internal, short, basic "nuclear localization signal" and mitochondrial matrix proteins that contain an N-terminal, amphipathic, α-helical "mitochondrial entry sequence."

VI. CO- AND POSTTRANSLATIONAL MODIFICATION OF POLYPEPTIDE CHAINS

Many polypeptide chains are covalently modified, either while they are still attached to the ribosome (cotranslational) or after their synthesis has been completed (posttranslational). These modifications may include removal of part of the translated sequence or the covalent addition of one or more chemical groups required for protein activity. Examples of such modifications are listed below.

A. Trimming

Many proteins destined for secretion from the cell are initially made as large, precursor molecules that are not functionally active. Portions of the protein chain must be removed by specialized endoproteases, resulting in the release of an active molecule. The cellular site of the cleavage reaction depends on the protein to be modified. Some precursor proteins are cleaved in the endoplasmic reticulum or the Golgi apparatus; others are cleaved in developing secretory vesicles (for example, insulin; see Figure 23.4, p. 309); and still others, such as collagen (see p. 47), are cleaved after secretion.

B. Covalent attachments

Proteins may be activated or inactivated by the covalent attachment of a variety of chemical groups (Figure 31.16). Examples include the following.

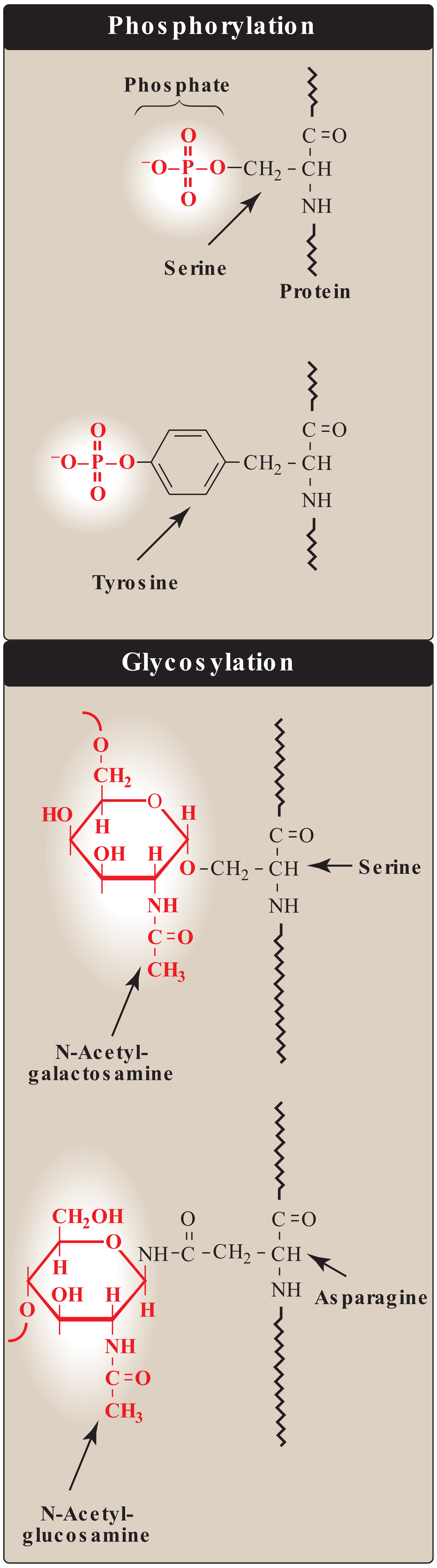


Figure 31.16 (continued on next page)
Covalent modifications of some amino acid residues.

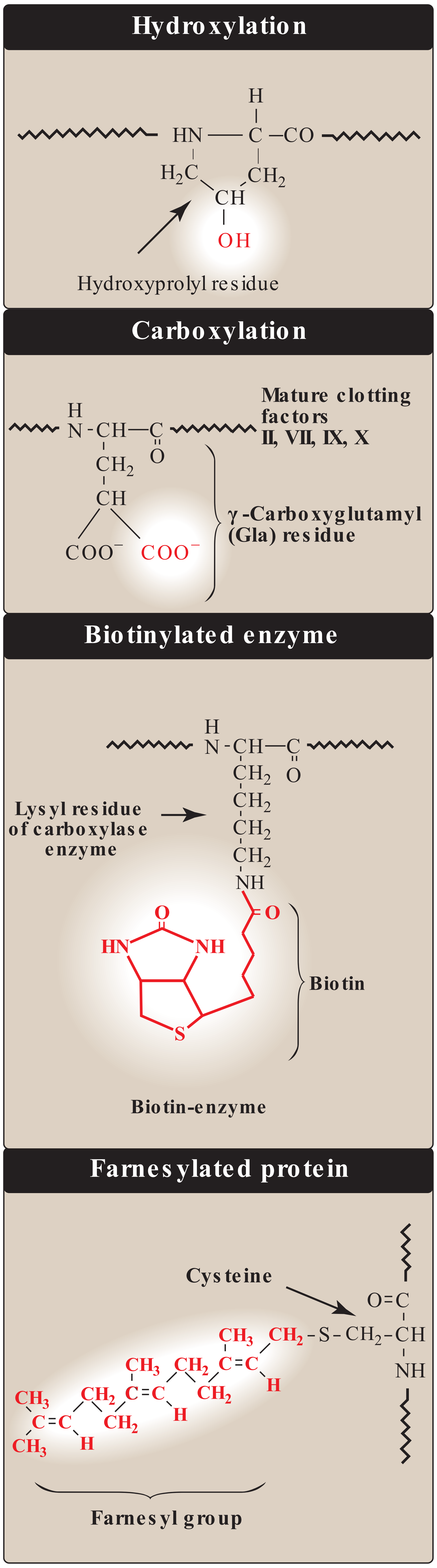


Figure 31.16 (continued from previous page)
Covalent modifications of some amino acid residues.

1. **Phosphorylation:** Phosphorylation occurs on the hydroxyl groups of serine; threonine; or, less frequently, tyrosine residues in a protein. This phosphorylation is catalyzed by one of a family of protein kinases and may be reversed by the action of cellular protein phosphatases. The phosphorylation may increase or decrease the functional activity of the protein. Several examples of phosphorylation reactions have been previously discussed (for example, see Chapter 11, p. 131, for the regulation of glycogen synthesis and degradation).

2. **Glycosylation:** Many of the proteins that are destined to become part of a plasma membrane or to be secreted from a cell have carbohydrate chains added en bloc to the amide nitrogen of asparagine (N-linked) or built sequentially on the hydroxyl groups of serine, threonine, or hydroxylysine (O-linked). N-glycosylation occurs in the endoplasmic reticulum and O-glycosyation in the Golgi. (The process of producing such glycoproteins was discussed on p. 165.) Glycosylation is also used to target proteins to the matrix of lysosomes. Lysosomal acid hydrolases are modified by the phosphorylation of mannose residues at carbon 6 (see p. 169).

3. **Hydroxylation:** Proline and lysine residues of the a chains of collagen are extensively hydroxylated by vitamin C–dependent hydroxylases in the endoplasmic reticulum (see p. 47).

4. **Other covalent modifications:** These may be required for the functional activity of a protein. For example, additional carboxyl groups can be added to glutamate residues by vitamin K–dependent carboxylation (see p. 389). The resulting g-carboxyglutamate (Gla) residues are essential for the activity of several of the blood-clotting proteins. (See online Chapter 34.) Biotin is covalently bound to the e-amino groups of lysine residues of biotin-dependent enzymes that catalyze carboxylation reactions such as pyruvate carboxylase (see p. 119). Attachment of lipids, such as farnesyl groups, can help anchor proteins to membranes. Many eukaryotic proteins are cotranslationally acetylated at the N-end. [Note: Reversible acetylation of histone proteins influences gene expression (see p. 409).]

C. Protein folding

Proteins must fold to assume their functional, native state. Folding can be spontaneous (as a result of the primary structure) or facilitated by proteins known as chaperones (see p. 20).

D. Protein degradation

Proteins that are defective (for example, misfolded) or destined for rapid turnover are often marked for destruction by ubiquitination, the attachment of chains of a small, highly conserved protein, called ubiquitin (see Figure 19.3 on p. 247). Proteins marked in this way are rapidly degraded by a cellular component known as the proteasome, which is a macromolecular, ATP-dependent, proteolytic system located in the cytosol. [Note: The DF508 mutation seen in CF causes misfolding of the CFTR protein, resulting in its proteasomal degradation.]

VII. CHAPTER SUMMARY

Codons are composed of three nucleotide bases presented in the messenger (mRNA) language of **adenine** (A), **guanine** (G), **cytosine** (C), and **uracil** (U). They are always written **5'→3'**. Of the 64 possible three-base combinations, 61 code for the 20 common amino acids and 3 signal termination of protein synthesis (**translation**). Altering the nucleotide sequence in a codon can cause **silent mutations** (the altered codon codes for the original amino acid), **missense mutations** (the altered codon codes for a different amino acid), or **nonsense mutations** (the altered codon is a termination codon). Characteristics of the genetic code include **specificity**, **universality**, and **degeneracy**, and it is **nonoverlapping** and **commaless** (Figure 31.17). Requirements for protein synthesis include all the amino acids that eventually appear in the finished protein, at least one specific type of transfer RNA (tRNA) for each amino acid, one aminoacyl-tRNA synthetase for each amino acid, the mRNA coding for the protein to be synthesized, fully competent **ribosomes**, **protein factors** needed for initiation, elongation, and termination of protein synthesis, and **ATP** and **GTP** as energy sources. tRNA has an attachment site for a specific amino acid at its 3'-end, and an anticodon region that can recognize the codon specifying the amino acid the tRNA is carrying. **Ribosomes** are large complexes of protein and ribosomal (rRNA). They consist of **two subunits**. Each ribosome has three binding sites for tRNA molecules: the A, P, and E sites that cover three neighboring codons. The **A-site** codon binds an **incoming aminoacyl-tRNA**, the **P-site** codon is occupied by **peptidyl-tRNA**, and the **E site** is occupied by the **empty tRNA** as it is about to exit the ribosome. Recognition of an mRNA codon is accomplished by the tRNA **anticodon**. The anticodon binds to the codon following the rules of **complementarity** and **antiparallel** binding. (Nucleotide sequences are always assumed to be written in the 5' to 3' direction unless otherwise noted.) The **"wobble" hypothesis** states that the first (5') base of the anticodon is not as spatially defined as the other two bases. Movement of that first base allows nontraditional base-pairing with the last (3') base of the codon, thus allowing a single tRNA to recognize more than one codon for a specific amino acid. For **initiation of protein synthesis**, the components of the translation system are assembled, and mRNA associates with the small ribosomal subunit. The process requires **initiation factors**. In **prokaryotes**, a purine-rich region of the mRNA (the **Shine-Dalgarno sequence**) base-pairs with a complementary sequence on 16S rRNA, resulting in the positioning of the small subunit on the mRNA so that translation can begin. The **5'-cap** (bound by proteins of the eIF-4 family) on **eukaryotic mRNA** is used to position the small subunit on the mRNA. The **initiation codon** is **AUG**, and **N-formylmethionine** is the initiating amino acid in prokaryotes, whereas methionine is used in eukaryotes. The polypeptide chain is elongated by the addition of amino acids to the carboxyl end of its growing chain. The process requires **elongation factors** that facilitate the binding of the aminoacyl-tRNA to the A site as well as the movement of the ribosome along the mRNA. The formation of the peptide bond is catalyzed by peptidyltransferase, which is an activity intrinsic to the rRNA of the large subunit and, therefore, is a **ribozyme**. Following peptide bond formation, the ribosome advances along the mRNA in the **5'→3' direction** to the next codon (translocation). Because of the length of most mRNAs, more than one ribosome at a time can translate a message, forming a **polysome**. Termination begins when one of the three termination codons moves into the A site. These codons are recognized by **release factors**. The newly synthesized protein is released from the ribosomal complex, and the ribosome is dissociated from the mRNA. Initiation, elongation, and termination are driven by the hydrolysis of **GTP**. Initiation in eukaryotes also requires **ATP** for scanning. Numerous **antibiotics** interfere with the process of protein synthesis. Many polypeptide chains are covalently modified during or after translation. Such modifications include **removal of amino acids**; **phosphorylation,** which may activate or inactivate the protein; **glycosylation**, which plays a role in protein targeting; and **hydroxylation** such as that seen in collagen. Proteins must fold to achieve their functional form. Folding can be spontaneous or facilitated by **chaperones**. Proteins that are defective (for example misfolded) or destined for rapid turnover are marked for destruction by the attachment of chains of a small, highly conserved protein called **ubiquitin**. Ubiquitinated proteins are rapidly degraded by a cytosolic complex known as the **proteasome**.

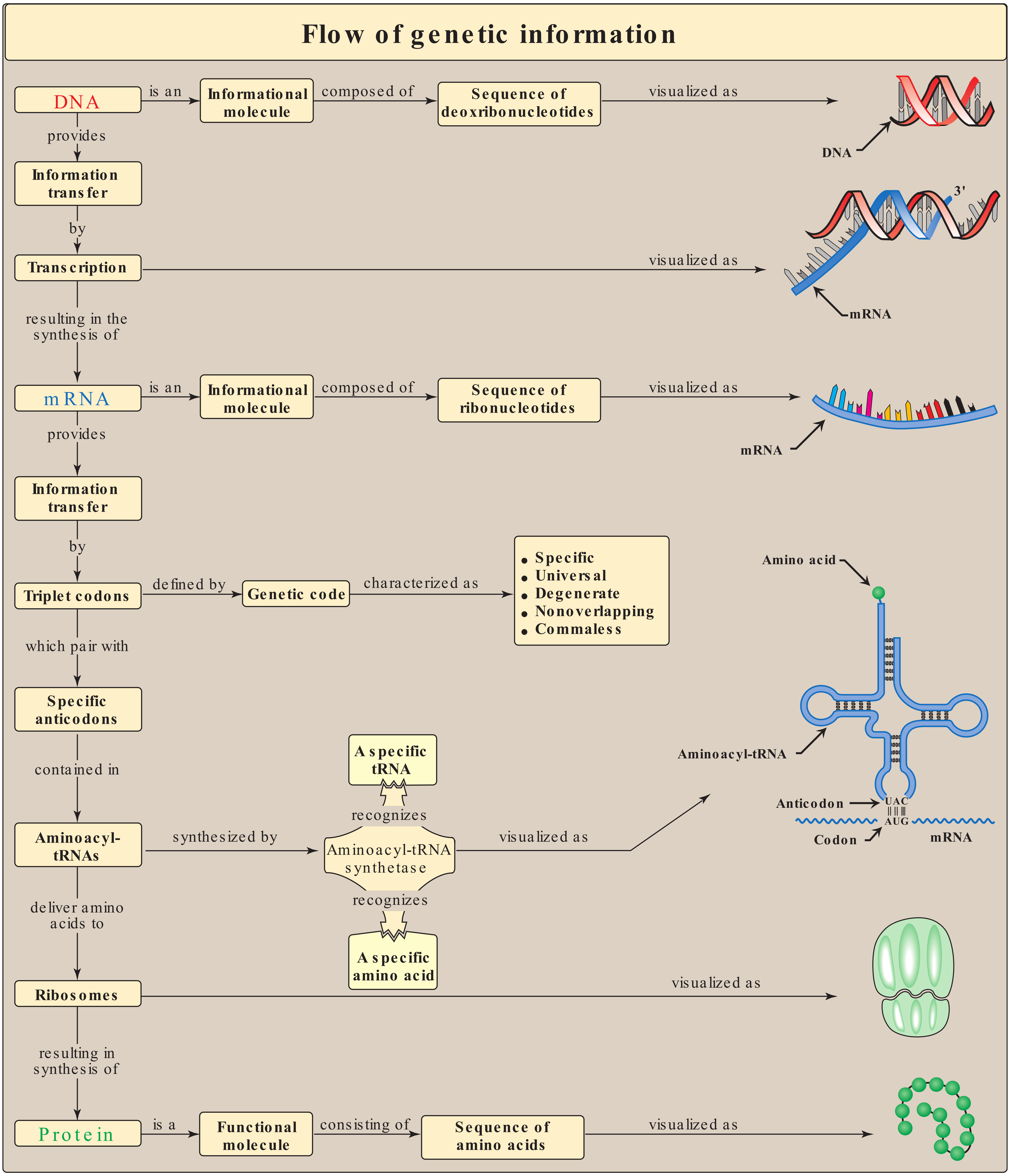

Flow of genetic information
DNA
is an
Informational molecule
composed of
Sequence of deoxribonucleotides
visualized as
DNA
provides
Information transfer
by
Transcription
visualized as
3'
mRNA
resulting in the synthesis of
mRNA
is an
Informational molecule
composed of
Sequence of ribonucleotides
visualized as
mRNA
provides
Information transfer
by
Triplet codons
defined by
Genetic code
characterized as
• Specific
• Universal
• Degenerate
• Nonoverlapping
• Commaless
Amino acid
which pair with
Specific anticodons
contained in
A specific tRNA
recognizes
Aminoacyl-tRNA
Anticodon
UAC
AUG
Codon
mRNA
Aminoacyl-tRNAs
synthesized by
Aminoacyl-tRNA synthetase
visualized as
recognizes
A specific amino acid
deliver amino acids to
Ribosomes
visualized as
resulting in synthesis of
Protein
is a
Functional molecule
consisting of
Sequence of amino acids
visualized as

Figure 31.17
Key concept map for protein synthesis. mRNA = messenger RNA; tRNA = transfer RNA; A = adenine; G = guanine; C = cytosine; U = uracil.

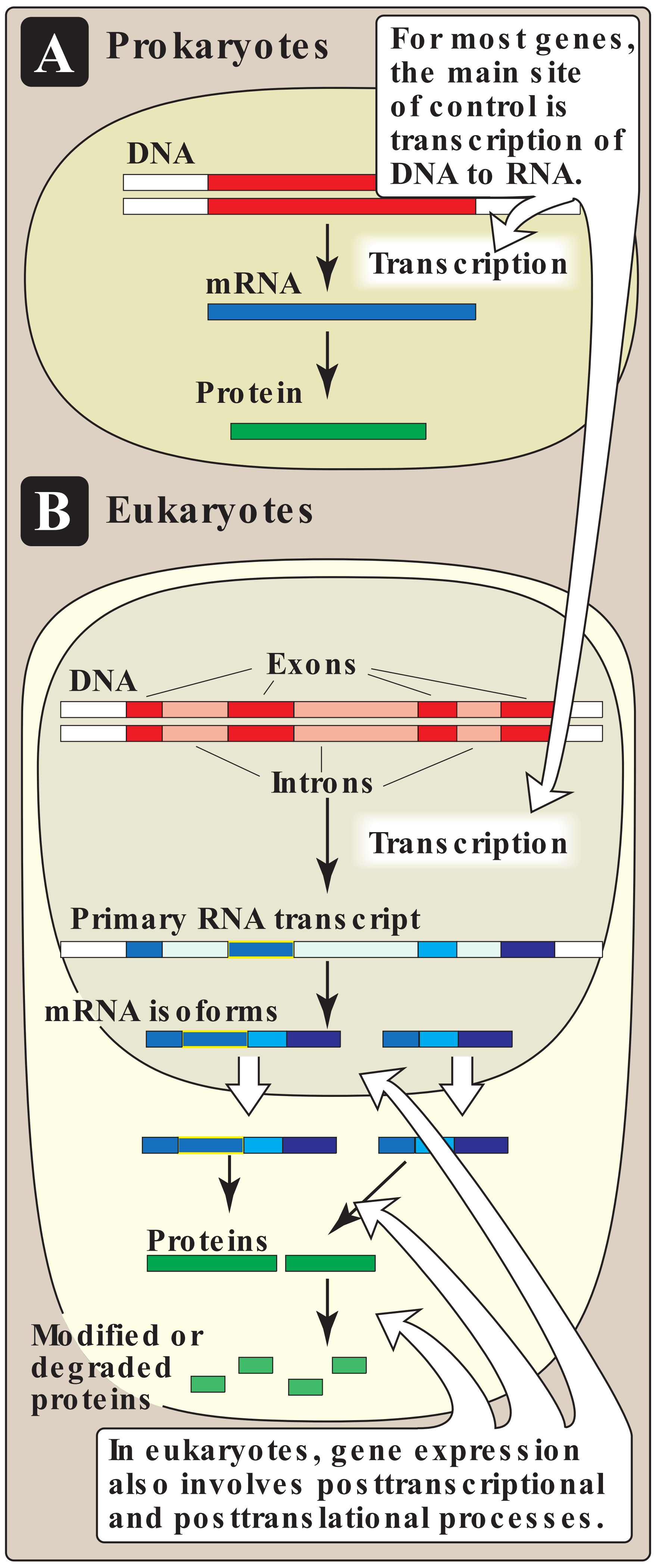
Regulation of Gene Expression

32

I. OVERVIEW

Gene expression refers to the multistep process that ultimately results in the production of a functional gene product, either ribonucleic acid (RNA) or protein. The first step in gene expression, the use of deoxyribonucleic acid (DNA) for the synthesis of RNA (transcription), is the primary site of regulation in both prokaryotes and eukaryotes. In eukaryotes, however, gene expression also involves extensive posttranscriptional and posttranslational processes as well as actions that influence access to particular regions of the DNA. Each of these steps can be regulated to provide additional control over the kinds and amounts of functional products that are produced.

Not all genes are regulated. For example, genes described as constitutive encode products required for basic cellular functions and so are continually expressed. They are also known as "housekeeping" genes. Regulated genes, however, are expressed only under certain conditions. They may be expressed in all cells or in only a subset of cells, for example, hepatocytes. The ability to regulate gene expression (that is, to determine if, how much, and when particular gene products will be made) gives the cell control over structure and function. It is the basis for cellular differentiation, morphogenesis, and adaptability of any organism. Control of gene expression is best understood in prokaryotes, but many themes are repeated in eukaryotes. Figure 32.1 lists some common strategies employed in gene regulation.



For most genes, the main site of control is transcription of DNA to RNA.

In eukaryotes, gene expression also involves posttranscriptional and posttranslational processes.

Figure 32.1
Control of gene expression. mRNA = messenger RNA.

II. REGULATORY SEQUENCES AND MOLECULES

Regulation of transcription, the initial step in all gene expression, is controlled by regulatory sequences of DNA, usually embedded in the noncoding regions of the genome. The interaction between these DNA segments and regulatory molecules, such as transcription factors, can engage or repress the transcriptional machinery, influencing the kinds and amounts of products that are produced. These DNA sequences flanking a gene are called cis-acting because they influence expression of genes only on the same chromosome (see p. 423). A trans-acting factor is the regulatory molecule itself, which can transit (diffuse) through the cell from its site of synthesis to its DNA-binding site (Figure 32.2). For example, a protein transcription factor (a trans-acting molecule) that regulates a gene on chromosome 6 might itself have been produced from a gene on chromosome 11. The binding of proteins to DNA is through structural motifs such as the zinc finger (Figure 32.3), leucine zipper, or helix-turn-helix in the protein. [Note: Some trans-acting factors can negatively affect gene expression.]

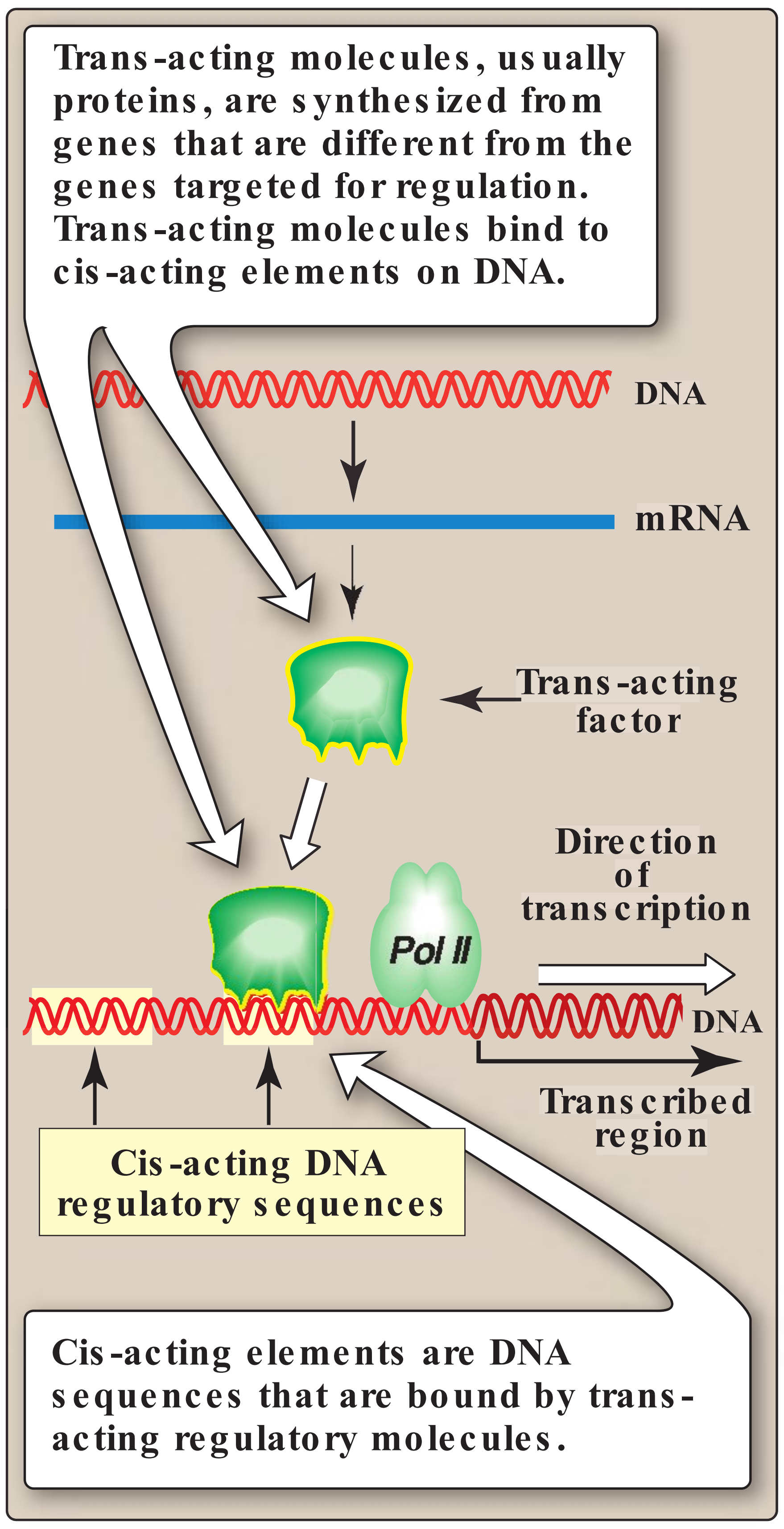

Trans-acting molecules, usually proteins, are synthesized from genes that are different from the genes targeted for regulation. Trans-acting molecules bind to cis-acting elements on DNA.
DNA
mRNA
Trans-acting factor
Direction of transcription
Pol II
DNA
Transcribed region
Cis-acting DNA regulatory sequences
Cis-acting elements are DNA sequences that are bound by trans-acting regulatory molecules.

Figure 32.2
Cis-acting elements and trans-acting molecules. mRNA = messenger RNA; Pol II = RNA polymerase II.

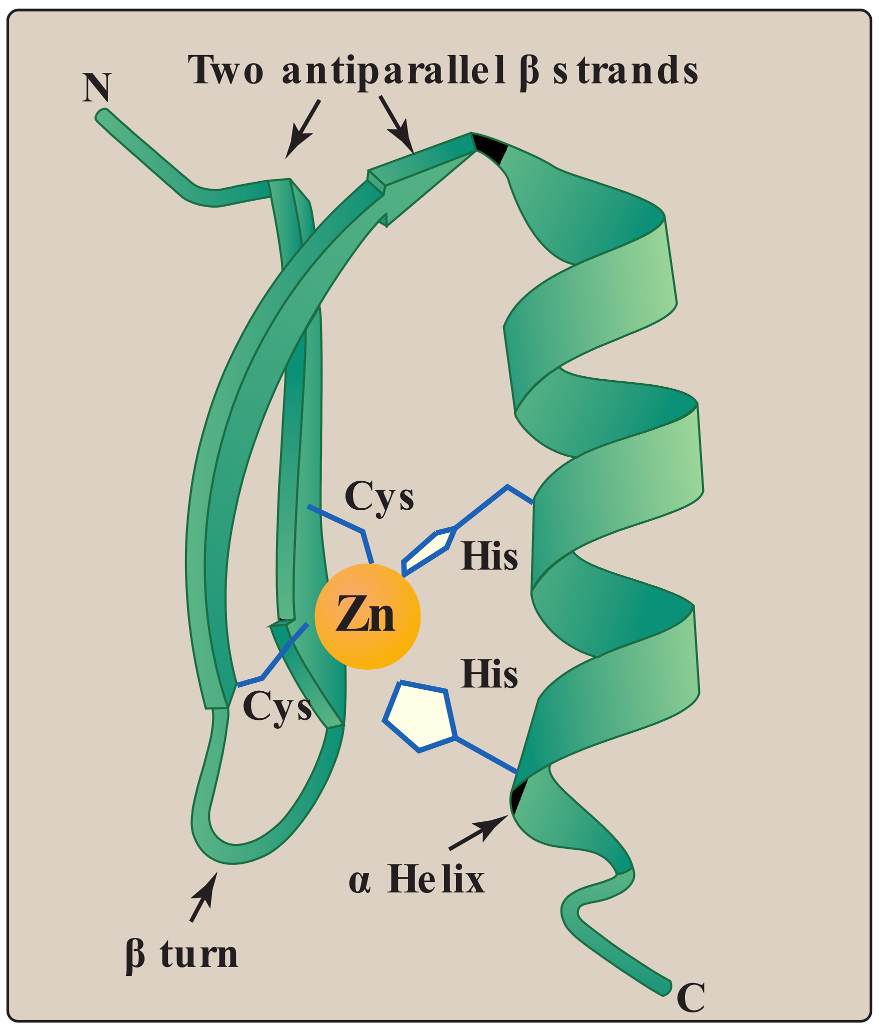

N
Two antiparallel β strands
Cys
His
Zn
His
Cys
α Helix
β turn
C

Figure 32.3
Zinc (Zn) finger is a common motif in proteins that bind DNA.
Cys = cysteine; His = histidine.

III. REGULATION OF PROKARYOTIC GENE EXPRESSION

In prokaryotes such as *Escherichia coli* (*E. coli*), regulation of gene expression occurs primarily at the level of transcription and, in general, is mediated by the binding of trans-acting proteins to cis-acting regulatory elements on their single DNA molecule (chromosome). [Note: Regulating the first step in the expression of a gene is an efficient approach, insofar as energy is not wasted making unneeded gene products.] Transcriptional control in prokaryotes can involve the initiation or premature termination of transcription.

A. Transcription of messenger RNA from bacterial operons

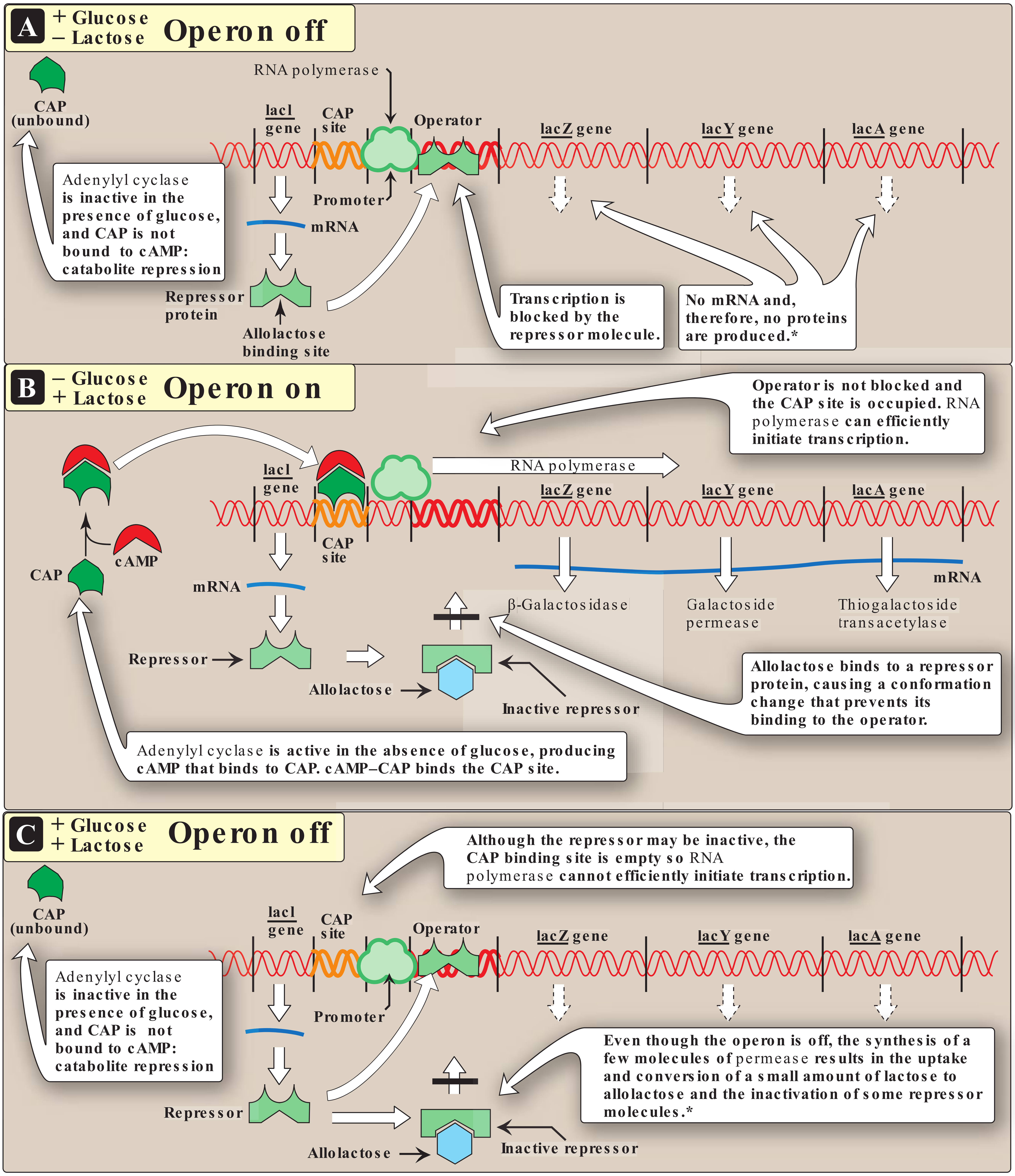
In bacteria, the structural genes that code for proteins involved in a particular metabolic pathway are often found sequentially grouped on the chromosome along with the cis-acting regulatory elements that determine the transcription of these genes. The transcription product is a single polycistronic messenger RNA (mRNA) (see p. 418). The genes are, thus, coordinately controlled (that is, turned on or off as a unit). This entire package is referred to as an operon.

B. Role of operators in prokaryotic transcription

Prokaryotic operons contain an operator, a segment of DNA that regulates the activity of the structural genes of the operon. If the operator is not bound by a repressor molecule, RNA polymerase passes over the operator and reaches the protein-coding genes which it transcribes to mRNA. If a repressor molecule is bound to the operator, the polymerase is blocked and does not produce mRNA. As long as the repressor is bound to the operator, no proteins are made. However, when an inducer molecule is present, it binds to the repressor, causing the repressor to change shape so that it no longer binds the operator. When this happens, the RNA polymerase can proceed with transcription. One of the best-understood examples is the inducible lactose operon of *E. coli* that illustrates both positive and negative regulation (Figure 32.4).

C. The lactose operon

The lactose (lac) operon contains the genes that code for three proteins involved in the catabolism of the disaccharide lactose: The *lacZ* gene codes for b-galactosidase, which hydrolyzes lactose to galactose and glucose; the *lacY* gene codes for a permease, which facilitates the movement of lactose into the cell; and the *lacA* gene codes for thiogalactoside transacetylase, which acetylates lactose.


A
+ Glucose
– Lactose
Operon off
CAP
(unbound)
RNA polymerase
lacI
gene
CAP
site
Operator
lacZ gene
lacY gene
lacA gene
Promoter
mRNA
Repressor
protein
Allolactose
binding site
B
– Glucose
+ Lactose
Operon on
cAMP
CAP
lacI
gene
CAP
site
RNA polymerase
lacZ gene
lacY gene
lacA gene
mRNA
mRNA
Repressor
Allolactose
Inactive repressor
β-Galactosidase
Galactoside
permease
Thiogalactoside
transacetylase
C
+ Glucose
+ Lactose
Operon off
CAP
(unbound)
lacI
gene
CAP
site
Operator
lacZ gene
lacY gene
lacA gene
Promoter
Repressor
Allolactose
Inactive repressor

Adenylyl cyclase is inactive in the presence of glucose, and CAP is not bound to cAMP: catabolite repression

Transcription is blocked by the repressor molecule.

No mRNA and, therefore, no proteins are produced.*

Operator is not blocked and the CAP site is occupied. RNA polymerase can efficiently initiate transcription.

Allolactose binds to a repressor protein, causing a conformation change that prevents its binding to the operator.

Adenylyl cyclase is active in the absence of glucose, producing cAMP that binds to CAP. cAMP–CAP binds the CAP site.

Although the repressor may be inactive, the CAP binding site is empty so RNA polymerase cannot efficiently initiate transcription.

Adenylyl cyclase is inactive in the presence of glucose, and CAP is not bound to cAMP: catabolite repression

Even though the operon is off, the synthesis of a few molecules of permease results in the uptake and conversion of a small amount of lactose to allolactose and the inactivation of some repressor molecules.*

Figure 32.4
The lactose operon of E. coli. *[Note: Even when the operon has been turned off by catabolite repression, the repressor transiently dissociates from the operator at a slow rate, allowing a very low level of expression. The synthesis of a few molecules of permease (and β-galactosidase) allows the organism to respond rapidly should glucose become unavailable.] CAP = catabolite activator protein; cAMP = cyclic adenosine monophosphate; mRNA = messenger RNA.

[Note: The physiologic function of this acetylation is unknown.] All of these proteins are maximally produced only when lactose is available to the cell and glucose is not. [Note: Bacteria use glucose, if available, as a fuel in preference to any other sugar.] The regulatory portion of the operon is upstream of the three structural genes and consists of the promoter region where RNA polymerase binds and two additional sites, the operator (O) site and the CAP site, where regulatory proteins bind. The lacZ, lacY, and lacA genes are expressed only when the O site is empty, and the CAP site is bound by a complex of cyclic adenosine monophosphate ([cAMP] see p. 94) and the catabolite activator protein (CAP), sometimes called the cAMP regulatory protein (CRP). A regulatory gene, the lacI gene, codes for the repressor protein (a trans-acting factor) that binds to the O site with high affinity. [Note: The lacI gene has its own promoter.]

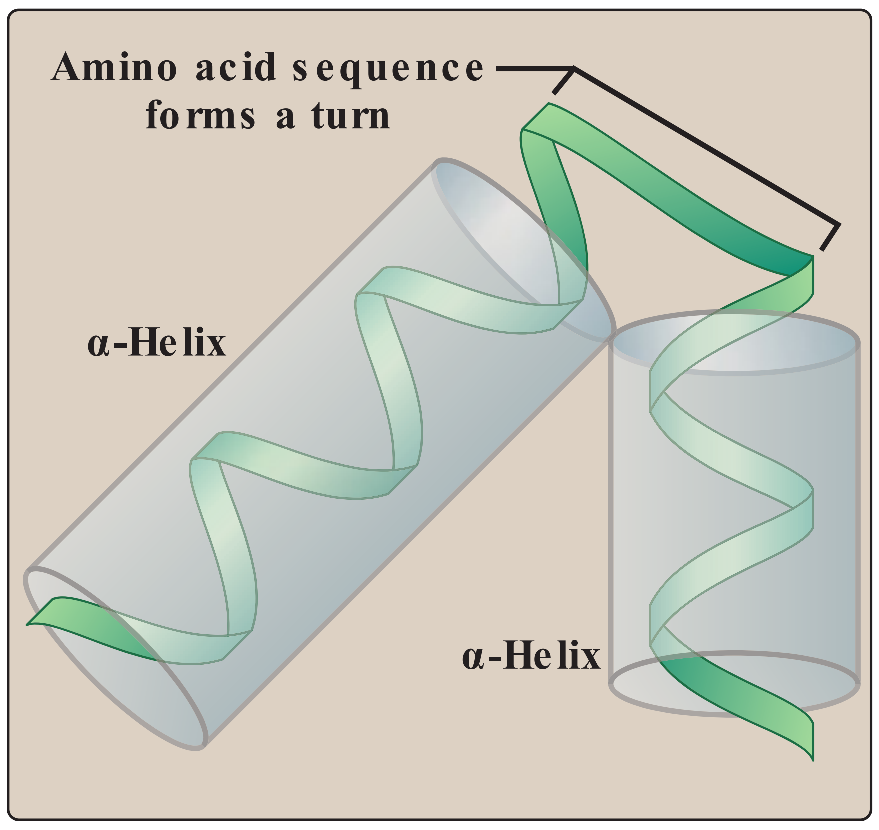


Figure 32.5
Helix-turn-helix motif of the lac repressor protein.

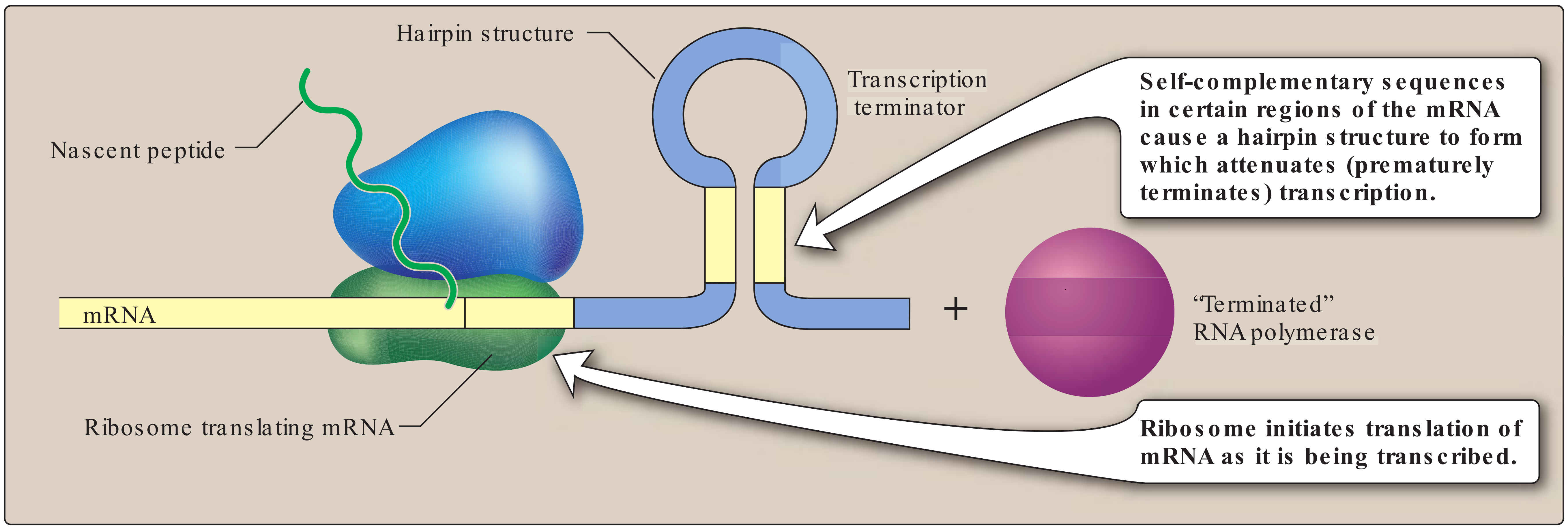
1. **When only glucose is available:** In this case, the lac operon is repressed (turned off). Repression is mediated by the repressor protein binding via a helix-turn-helix motif (Figure 32.5) to the operator site, which is downstream of the promoter region (see Figure 32.4A). Binding of the repressor interferes with the progress of RNA polymerase and blocks transcription of the structural genes. This is an example of negative regulation.

2. **When only lactose is available:** In this case, the lac operon is induced (maximally expressed, or turned on). A small amount of lactose is converted to an isomer, allolactose. This compound is an inducer that binds to the repressor protein, changing its conformation so that it can no longer bind to the operator. In the absence of glucose, adenylyl cyclase is active, and sufficient quantities of cAMP are made and bind to the CAP protein. The cAMP–CAP trans-acting complex binds to the CAP site, causing RNA polymerase to more efficiently initiate transcription at the promoter site (see Figure 32.4B). This is an example of positive regulation. The transcript is a single polycistronic mRNA molecule that contains three sets of start and stop codons. Translation of the mRNA produces the three proteins that allow lactose to be used for energy production by the cell. [Note: In contrast to the inducible lacZ, lacY, and lacA genes, whose expression is regulated, the lacI gene is constitutive. Its gene product, the repressor protein, is always made and is active unless the inducer is present.]

3. **When both glucose and lactose are available:** In this case, transcription of the lac operon is negligible, even if lactose is present at a high concentration. Adenylyl cyclase is inhibited in the presence of glucose (a process known as catabolite repression) so no cAMP–CAP complex forms, and the CAP site remains empty. RNA polymerase is, therefore, unable to effectively initiate transcription, even though the repressor may not be bound to the operator region. Consequently, the three structural genes of the operon are not expressed (see Figure 32.4C).

D. Tryptophan operon

The tryptophan (trp) operon contains five structural genes that code for enzymes required for the synthesis of the amino acid, tryptophan.



Self-complementary sequences in certain regions of the mRNA cause a hairpin structure to form which attenuates (prematurely terminates) transcription.

Ribosome initiates translation of mRNA as it is being transcribed.

Figure 32.6
Attenuation of transcription of the trp operon when tryptophan is plentiful. mRNA = messenger RNA.

As with the lac operon, the trp operon is subject to negative control. However, for the repressible trp operon, negative control includes Trp itself binding to a repressor protein and facilitating the binding of the repressor to the operator: Trp is a corepressor. Because repression by Trp is not always complete, unlike the lac operon, the trp operon is also regulated by a process known as attenuation. With attenuation, transcription is initiated but is terminated well before completion (Figure 32.6). If Trp is plentiful, transcription initiation that escaped repression by Trp is attenuated (stopped) by the formation at the 5'-end of the mRNA of a hairpin (stem–loop) structure like that seen in rho-independent termination (see p. 421). [Note: Transcription and translation are temporally linked in prokaryotes (see p. 438), and, therefore, attenuation also results in the formation of a truncated, nonfunctional peptide product that is rapidly degraded.] If Trp becomes scarce, the operon is expressed. The 5'-end of the mRNA contains two adjacent codons for Trp. The lack of Trp causes ribosomes to stall at these codons, covering regions of the mRNA required for formation of the attenuation hairpin. This prevents attenuation and allows transcription to continue.

> Transcriptional attenuation can occur in prokaryotes because translation of an mRNA begins before its synthesis is complete. This does not occur in eukaryotes because the presence of a membrane-bound nucleus spatially and temporally separates transcription and translation.

E. Coordination of transcription and translation in prokaryotes

Whereas transcriptional regulation of mRNA production is primary in bacteria, regulation at the level of ribosomal RNA (rRNA) and protein synthesis also occurs and plays important roles in the microbe's ability to adapt to environmental stress.

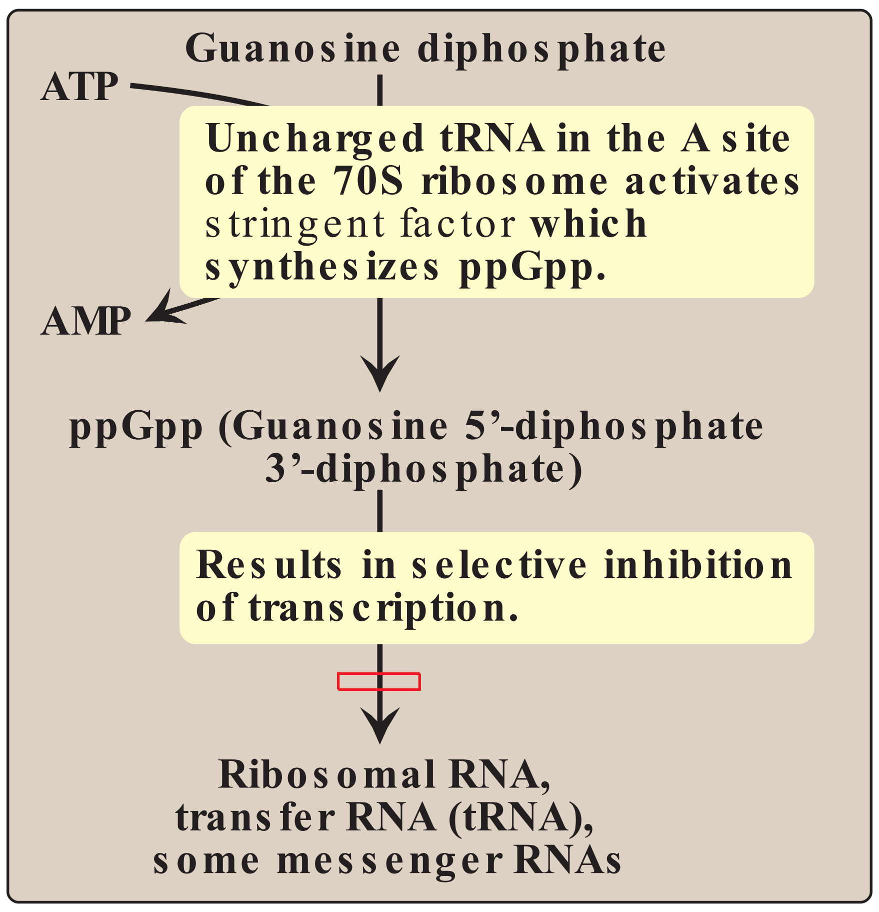

Guanosine diphosphate
ATP
Uncharged tRNA in the A site of the 70S ribosome activates stringent factor which synthesizes ppGpp.
AMP
ppGpp (Guanosine 5'-diphosphate 3'-diphosphate)
Results in selective inhibition of transcription.
Ribosomal RNA, transfer RNA (tRNA), some messenger RNAs

Figure 32.7
Regulation of transcription by the stringent response to amino acid starvation. S = Svedberg unit.

1. **Stringent response:** E. coli has seven operons that synthesize the rRNA needed for ribosome assembly, and each is regulated in response to changes in environmental conditions. Regulation in response to amino acid starvation is known as the stringent response. The binding of an uncharged transfer RNA (tRNA) to the A site of a ribosome (see p. 436) triggers a series of events that leads to the production of a polyphosphorylated guanosine, ppGpp. The synthesis of this unusual derivative of guanosine diphosphate (GDP) is catalyzed by stringent factor (RelA), an enzyme physically associated with ribosomes. Elevated levels of ppGpp result in inhibition of rRNA synthesis (Figure 32.7). [Note: In addition to rRNA, tRNA synthesis and some mRNA synthesis (for example, for ribosomal proteins) are also inhibited. However, synthesis of mRNAs for enzymes required for amino acid biosynthesis is not inhibited. ppGpp appears to alter promoter selection through use of different sigma-factors for RNA polymerase (see p.419.]

2. **Regulatory ribosomal proteins:** Operons for ribosomal proteins (r-proteins) can be inhibited by an excess of their own protein products. For each operon, one specific r-protein functions in the repression of translation of the polycistronic mRNA from that operon (Figure 32.8). The r-protein does so by binding to the Shine-Dalgarno (SD) sequence located on the mRNA just upstream of the first initiating AUG codon (see p. 439) and acting as a physical impediment to the binding of the small ribosomal subunit to the SD sequence. One r-protein thus inhibits synthesis of all the r-proteins of the operon. This same r-protein also binds to rRNA and with a higher affinity than for mRNA. If the concentration of rRNA falls, the r-protein then is available to bind its own mRNA and inhibit its translation. This coordinated regulation keeps the synthesis of r-proteins in balance with the transcription of rRNA, so that each is present in appropriate amounts for the formation of ribosomes.

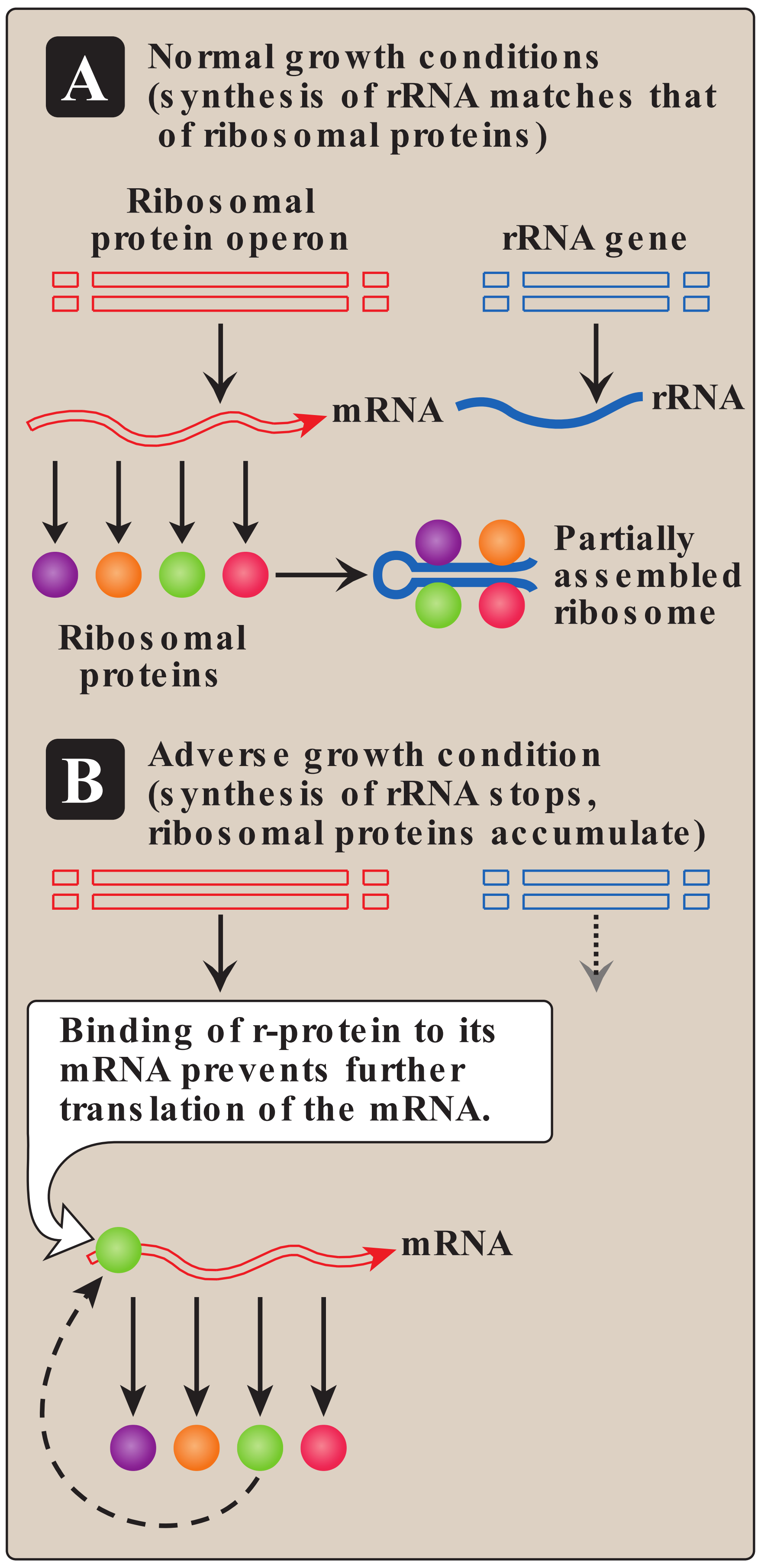
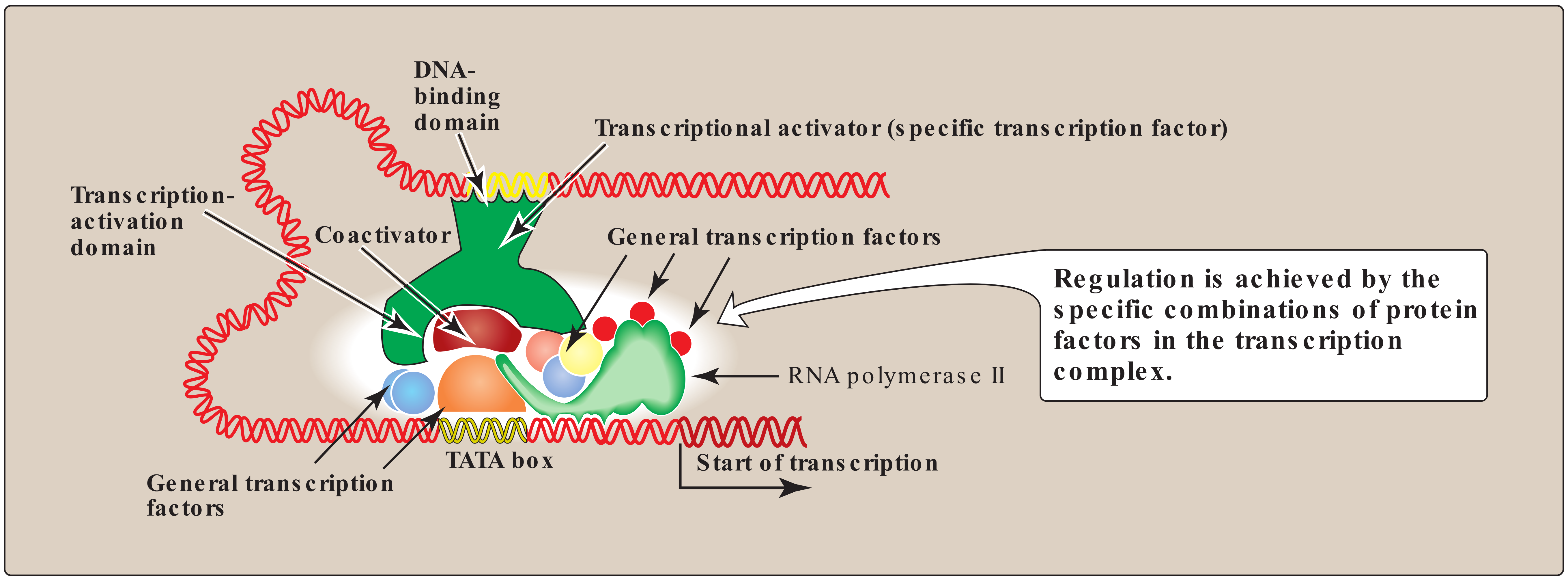

A
Normal growth conditions (synthesis of rRNA matches that of ribosomal proteins)
Ribosomal protein operon
rRNA gene
mRNA
rRNA
Partially assembled ribosome
Ribosomal proteins
B
Adverse growth condition (synthesis of rRNA stops, ribosomal proteins accumulate)
Binding of r-protein to its mRNA prevents further translation of the mRNA.
mRNA

Figure 32.8
Regulation of translation by an excess of ribosomal proteins. mRNA = messenger RNA; rRNA = ribosomal RNA; r-protein = ribosomal protein.

IV. REGULATION OF EUKARYOTIC GENE EXPRESSION

The higher degree of complexity of eukaryotic genomes, as well as the presence of a nuclear membrane, necessitates a wider range of regulatory processes. As with the prokaryotes, the primary site of regulation is at the level of transcription. Again, the theme of trans-acting molecules binding to cis-acting elements is seen. Operons, however, are generally not found in eukaryotes, which must use alternative strategies to solve the problem of how to coordinately regulate all the genes required for a specific response. In eukaryotes, gene expression is also regulated at multiple levels other than transcription. For example, the major modes of posttranscriptional regulation at the mRNA level are alternative mRNA splicing, control of mRNA stability, and control of translational efficiency. Additional regulation at the protein level occurs by mechanisms that modulate stability, processing, or targeting of the protein.


DNA-binding domain
Transcriptional activator (specific transcription factor)
Transcription-activation domain
Coactivator
General transcription factors
RNA polymerase II
TATA box
Start of transcription
General transcription factors

Regulation is achieved by the specific combinations of protein factors in the transcription complex.

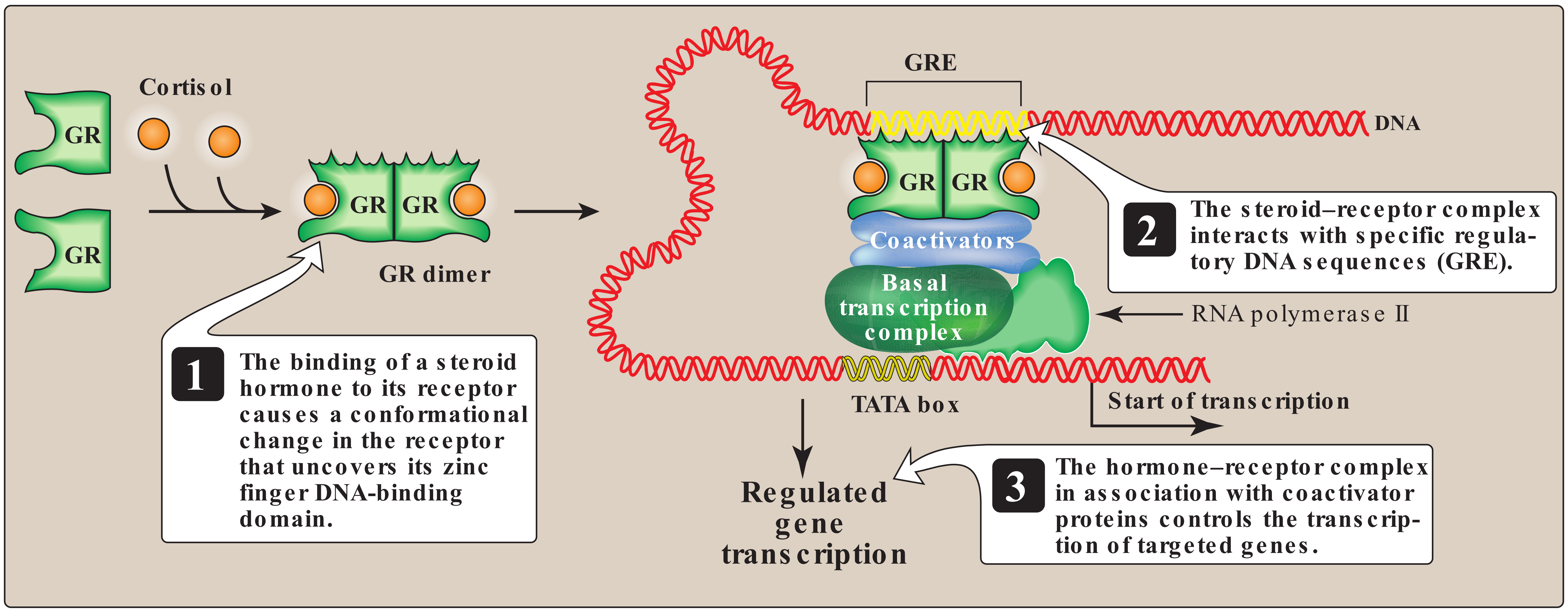
Figure 32.9
Combinatorial control of transcription.

A. Trans-acting molecules

Specific transcription factors are trans-acting DNA-binding proteins that function as transcriptional activators. They have at least two binding domains: the DNA-binding domain, and the transcription-activation domain. The DNA-binding domain contains specific structural motifs, such as zinc fingers (see p. 450), that bind consensus sequences in DNA. The transcription-activation domain recruits other proteins, such as the general transcription factors ([GTFs] see p.423) and coactivators (for example, histone acetyltransferases [HATs]; see p. 422). These facilitate formation of the transcription initiation complex (RNA polymerase II plus the GTFs) at the promoter, and, thus, activate transcription (Figure 32.9). Regulation is achieved by the formation of a multiprotein complex bound to DNA, with protein–protein and protein–DNA interactions controlling assembly of the complex. Although activation domains recruit a variety of proteins, the specific effect of any one of them is dependent upon the protein composition of the complex. This is known as combinatorial control. [Note: DNA-binding proteins can also inhibit transcription.]

B. Cis-acting regulatory elements

The need to coordinately regulate a group of genes to cause a particular response is of key importance in multicellular organisms including humans. An underlying theme occurs repeatedly: A protein binds to a regulatory consensus element on each of the genes in the group and coordinately affects the expression of those genes, even if they are on different chromosomes. For example, hormone-response elements (HREs) are cis-acting DNA sequences that bind trans-acting protein factors and regulate gene expression in response to hormonal signals. In general, hormones bind either to intracellular receptors (steroid hormones are an example; see p. 240) or to cell-surface receptors (the peptide hormone glucagon is an example; see p. 314).

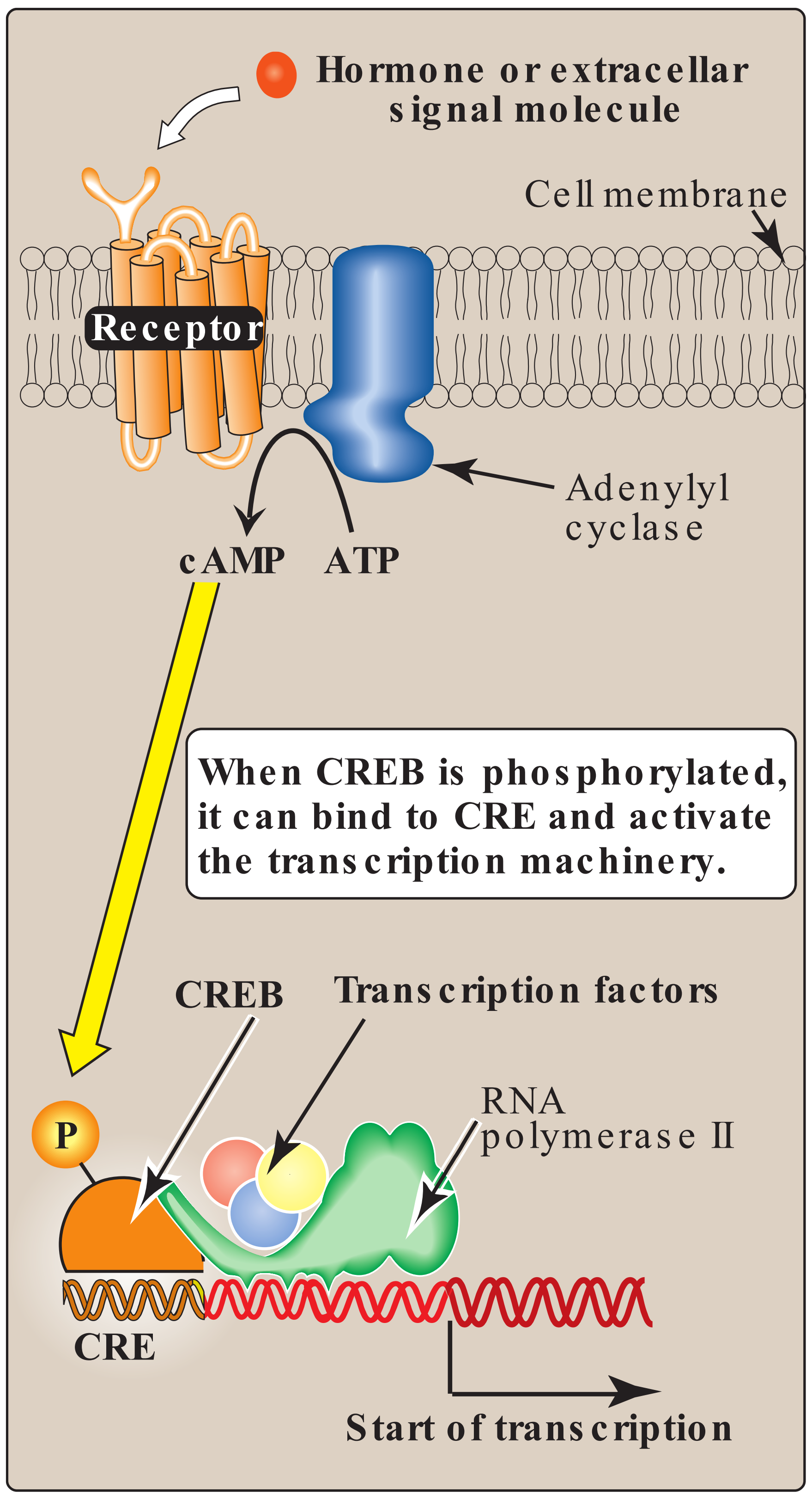

Cortisol
GR
GR
GR GR
GR dimer
GRE
DNA
GR GR
Coactivators
Basal transcription complex
RNA polymerase II
TATA box
Start of transcription
Regulated gene transcription

1 The binding of a steroid hormone to its receptor causes a conformational change in the receptor that uncovers its zinc finger DNA-binding domain.

2 The steroid–receptor complex interacts with specific regulatory DNA sequences (GRE).

3 The hormone–receptor complex in association with coactivator proteins controls the transcription of targeted genes.

Figure 32.10
Transcriptional regulation by intracellular steroid hormone receptors. GRE = glucocorticoid-response element (an example of a hormone-response element); GR = glucocorticoid receptor.


Hormone or extracellar signal molecule
Cell membrane
Receptor
Adenylyl cyclase
cAMP
ATP
CREB
Transcription factors
RNA polymerase II
P
CRE
Start of transcription

When CREB is phosphorylated, it can bind to CRE and activate the transcription machinery.

Figure 32.11
Transcriptional regulation by receptors located in the cell membrane. [Note: Cyclic AMP activates protein kinase A that phosphorylates cAMP response element–binding (CREB) protein.] CRE = cAMP response element.

1. **Regulatory signals mediated by intracellular receptors:** Members of the nuclear receptor superfamily, which includes the steroid hormone (glucocorticoids, mineralocorticoids, androgens, and estrogens), vitamin D, retinoic acid, and thyroid hormone receptors, all directly influence gene expression by functioning as specific transcription factors. These receptors, therefore, contain a DNA-binding domain and an activation domain. They also contain a ligand-binding domain. For example, steroid hormones such as cortisol (a glucocorticoid) bind to soluble, intracellular receptors at the ligand-binding domain (Figure 32.10). Binding causes a conformational change in the receptor that activates it. The receptor–ligand complex enters the nucleus, dimerizes, and binds via a zinc finger motif to nuclear DNA at a cis-acting regulatory element, the glucocorticoid-response element (GRE), an example of an HRE. Binding allows recruitement of coactivators to the activation domain and results in increased expression of cortisol-responsive genes, each of which is under the control of its own GRE. Binding of the receptor–hormone complex to the GRE allows coordinate expression of a group of target genes, even when these genes are located on different chromosomes. The GRE can be located upstream or downstream of the genes it regulates and is able to function at great distances from those genes. The GRE, then, can function as a true enhancer (see p. 424). [Note: If associated with corepressors, hormone–receptor complexes inhibit transcription.]

2. **Regulatory signals mediated by cell-surface receptors:** Cell-surface receptors include those for insulin, epinephrine, and glucagon. Glucagon, for example, is a peptide hormone that binds its G protein–coupled plasma membrane receptor on glucagon-responsive cells. This extracellular signal is then transduced to intracellular cAMP (Figure 32.11; also see Figure 8.7 on p. 95), which can affect protein expression (and activity) through protein kinase A–mediated phosphorylation. In response to a rise

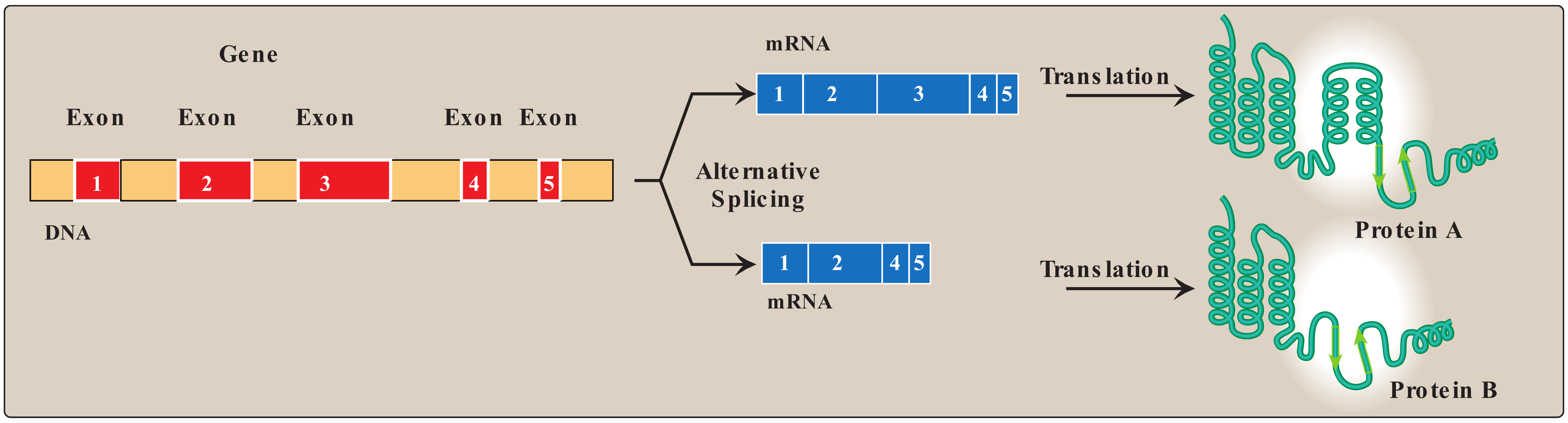


Figure 32.12
Tissue-specific alternative splicing produces multiple related proteins, or isoforms, from a single gene.

in cAMP, a trans-acting factor (cAMP response element–binding [CREB] protein) is phosphorylated and activated. Active CREB protein binds via a leucine zipper motif to a cis-acting regulatory element, the cAMP response element (CRE), resulting in transcription of target genes with CREs in their promoters. [Note: The genes for phosphoenolpyruvate carboxykinase and glucose 6-phosphatase, key enzymes of gluconeogenesis (see p. 117), are examples of genes upregulated by the cAMP/CRE/CREB system.]

C. Regulation by processing of messenger RNA

Eukaryotic mRNA undergoes several modifications before it is exported from the nucleus to the cytoplasm for use in protein synthesis (see p. 418). Capping at the 5'-end, polyadenylation at the 3'-end, and splicing are essential processing events for the production of a functional eukaryotic messenger from most pre-mRNA (see p. 425), and variations in these events can affect gene expression. In addition, messenger stability also affects gene expression.

1. **Splice-site choice:** Tissue-specific protein isoforms can be made from the same pre-mRNA through differential cotranscriptional processing, particularly the use of alternative splice sites (Figure 32.12). For example, tropomyosin (TM) is an actin filament–binding protein that regulates the functions of actin in both muscle and nonmuscle cells. Its pre-mRNA undergoes tissue-specific differential splicing to yield a number of TM isoforms (see p. 427).

> Over 60% percent of the approximately 25,000 genes in the human genome undergo differential splicing. The use of alternative polyadenylation and transcription start sites is also seen in many genes. This explains, at least in part, how 25,000 genes can give rise to hundreds of thousands of proteins.

2. **Messenger RNA editing:** Even after mRNA has been fully processed, it may undergo additional posttranscriptional modification in which a base in the mRNA is altered. This is known as

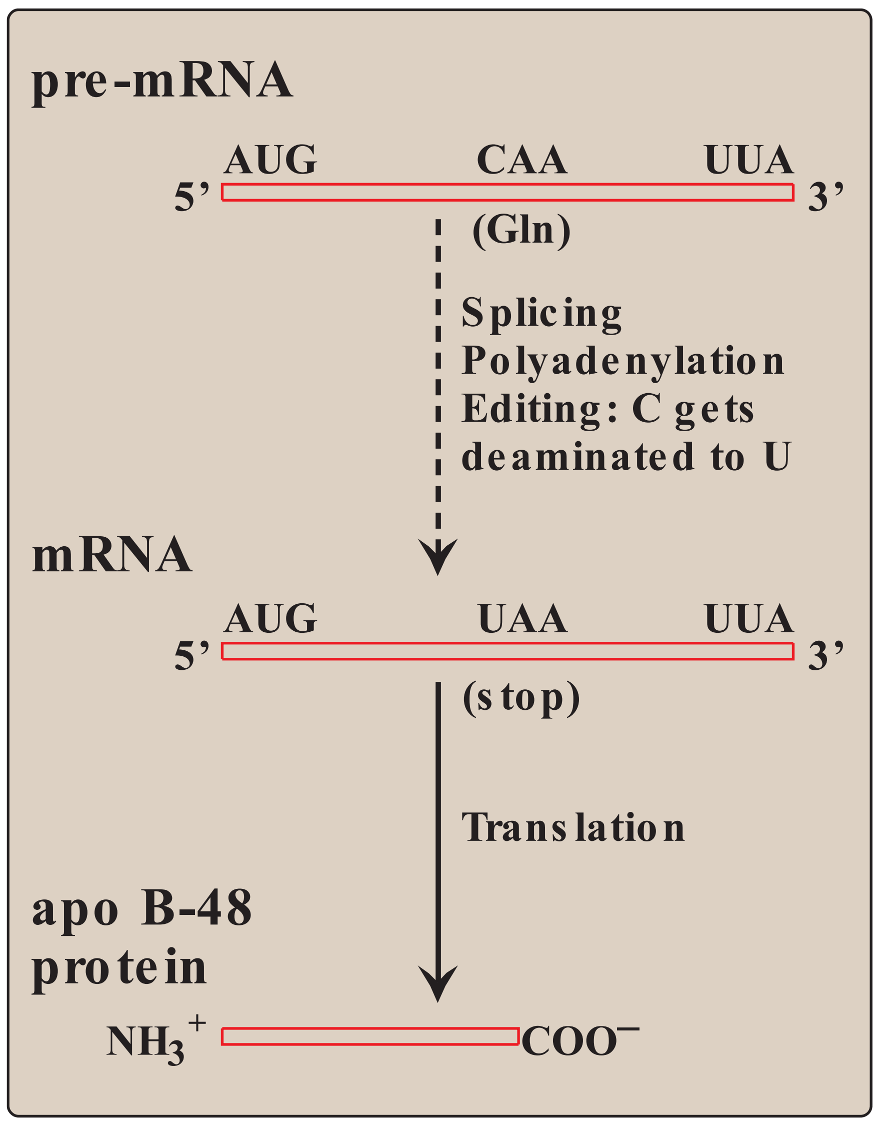

pre-mRNA
AUG
CAA
UUA
5'
3'
(Gln)
Splicing
Polyadenylation
Editing: C gets deaminated to U
mRNA
AUG
UAA
UUA
5'
3'
(stop)
Translation
apo B-48 protein
NH3+
COO−

Figure 32.13
RNA editing of apolipoprotein (apo) B in the intestine and generation of the apo B-48 protein needed for chylomicron synthesis. Gln = glutamine; mRNA = messenger RNA.

RNA editing. An important example in humans occurs with the transcript for apolipoprotein (apo) B, an essential component of chylomicrons (see p. 228) and very low density lipoproteins ([VLDL] see p. 231). Apo B mRNA is made in the liver and the small intestine. However, in the intestine only, the C residue in the CAA codon for glutamine is deaminated to U, changing the sense codon to a nonsense or stop codon (UAA), as shown in Figure 32.13. This results in a shorter protein (apo B-48, representing 48% of the message) being made in the intestine (and incorporated into chylomicrons) than is made in the liver (apo B-100, full-length, incorporated into VLDL).

3. **Messenger RNA stability:** How long an mRNA remains in the cytosol before it is degraded influences how much protein product can be produced from it. Regulation of iron metabolism and the gene-silencing process of RNA interference illustrate the importance of mRNA stability in the regulation of gene expression.

    a. **Iron metabolism:** Transferrin is a plasma protein that transports iron. Transferrin binds to cell-surface receptors (transferrin receptors [TfRs]) that get internalized and provide cells, such as erythroblasts, with iron. The mRNA for the TfR has several cis-acting iron-responsive elements (IREs) at its 3'-end. IREs have a short stem–loop structure that can be bound by trans-acting iron regulatory proteins ([IRPs] Figure 32.14). When the iron concentration in the cell is low, the IRPs bind to the 3'-IREs and stabilize the mRNA for TfR, allowing TfR synthesis. When intracellular iron levels are high, the IRPs are degraded. The lack of IRPs bound to the mRNA hastens its destruction, resulting in decreased TfR synthesis. [Note: The mRNA for apoferritin, an intracellular protein of iron storage, has a single IRE at its 5'-end. When iron levels in the cell are low, IRPs bind the 5'-IRE and prevent the use of the

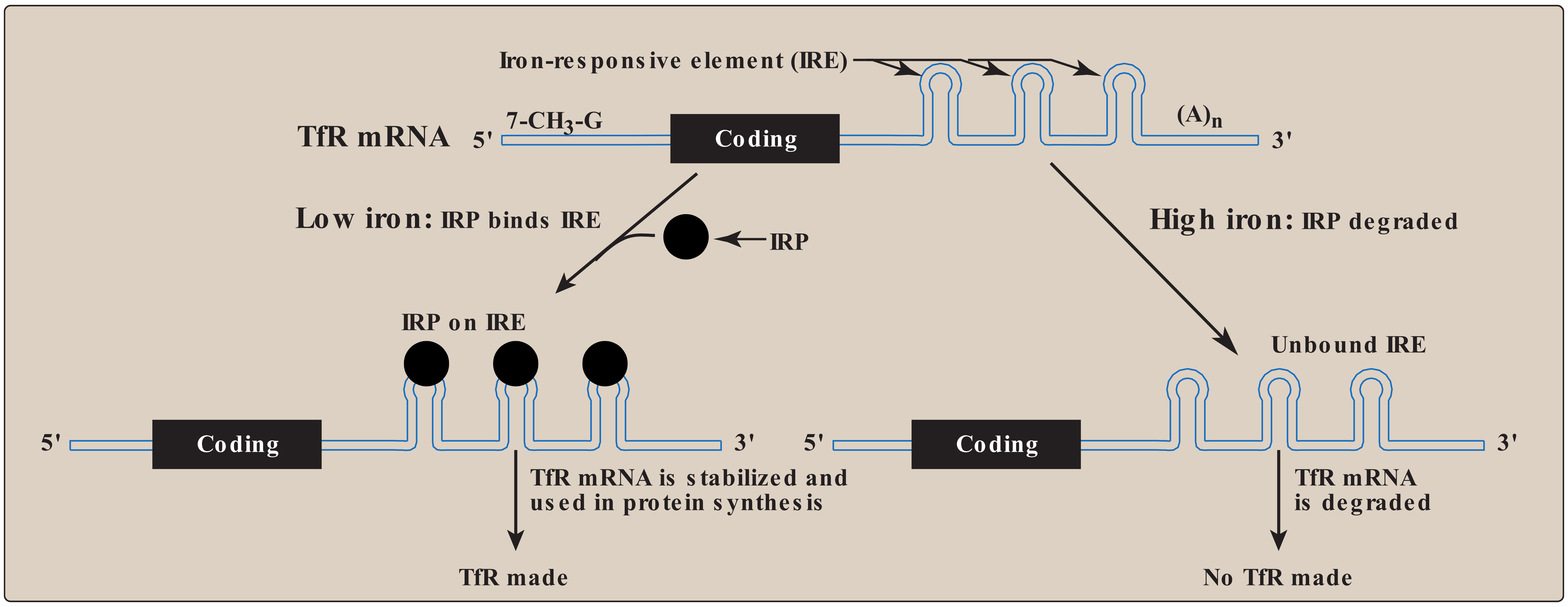

Iron-responsive element (IRE)
TfR mRNA
5'
7-CH3-G
Coding
(A)n
3'
Low iron: IRP binds IRE
IRP
High iron: IRP degraded
IRP on IRE
Unbound IRE
5'
Coding
3'
5'
Coding
3'
TfR mRNA is stabilized and used in protein synthesis
TfR mRNA is degraded
TfR made
No TfR made

Figure 32.14
Regulation of transferrin receptor (TfR) synthesis. IRP = iron regulatory protein. [Note: The IREs are located in the 3' UTR (untranslated region) of the TfR mRNA.]

mRNA, and less apoferritin is made. When iron accumulates in the cell, the IRP is degraded, allowing synthesis of apoferritin molecules to store the excess iron. ALAS2, the regulated enzyme of heme synthesis (see p. 279) in erythroblasts, also contains a 5'-IRE.]

b. RNA interference: RNA interference (RNAi) is a mechanism of gene silencing through decreased expression of mRNA, either by repression of translation or by increased degradation. It is thought to play a key role in such fundamental processes as cell proliferation, differentiation, and apoptosis. RNAi is mediated by short (~22 bp), noncoding RNAs called microRNAs (miRNAs), which arise from far longer, genomically encoded nuclear transcripts, primary miRNA (pri-miRNA) that are partially processed in the nucleus to pre-miRNA by an endonuclease (Drosha) then transported to the cytoplasm. There, an endonuclease (Dicer) completes the processing and generates short, double-stranded miRNA. A single strand (the guide, or antisense strand) of the miRNA associates with a cytosolic protein complex known as the RNA-induced silencing complex (RISC). The guide strand hybridizes with a complementary sequence on a full length target mRNA, bringing RISC to the mRNA. This can result in repression of translation of the mRNA or its degradation by an endonuclease (Argonaute/Ago/Slicer) of the RISC. The extent of complementarity appears to be the determining factor (Figure 32.15). RNAi can also be triggered by double-stranded short interfering RNAs (siRNAs) introduced into a cell from exogenous sources. [Note: In vertebrates, the function of siRNAs that may arise from endogenous sources is unclear.]

1) RNA-interference–based therapeutics: Modulation of gene expression by providing siRNA to trigger RNAi has enormous therapeutic potential. The first clinical trial of RNAi-based therapy involved patients with the neovascular form of age-related macular degeneration (AMD), a leading form of adult blindness. Neovascular AMD is triggered by overproduction of vascular endothelial growth factor (VEGF), leading to the sprouting of excess blood vessels behind the retina. The vessels leak, clouding and often entirely destroying vision (therefore, neovascular AMD is also referred to as "wet" macular degeneration). An siRNA designed to target the mRNA of VEGF and promote its degradation went to clinical trials. Although considerable effort and resources have been expended to develop RNAi-based therapeutics, especially for the treatment of cancer, no products have gone from trials to the market. Development has been hindered by the problems of targeted delivery and stability. The use of nano-sized vectors such as liposomes may eliminate these issues. The research applications of RNAi, however, have grown rapidly.

4. Translation of messenger RNA: Regulation of gene expression can also occur at the level of translation. One mechanism by which translation is regulated is through phosphorylation of the eukaryotic

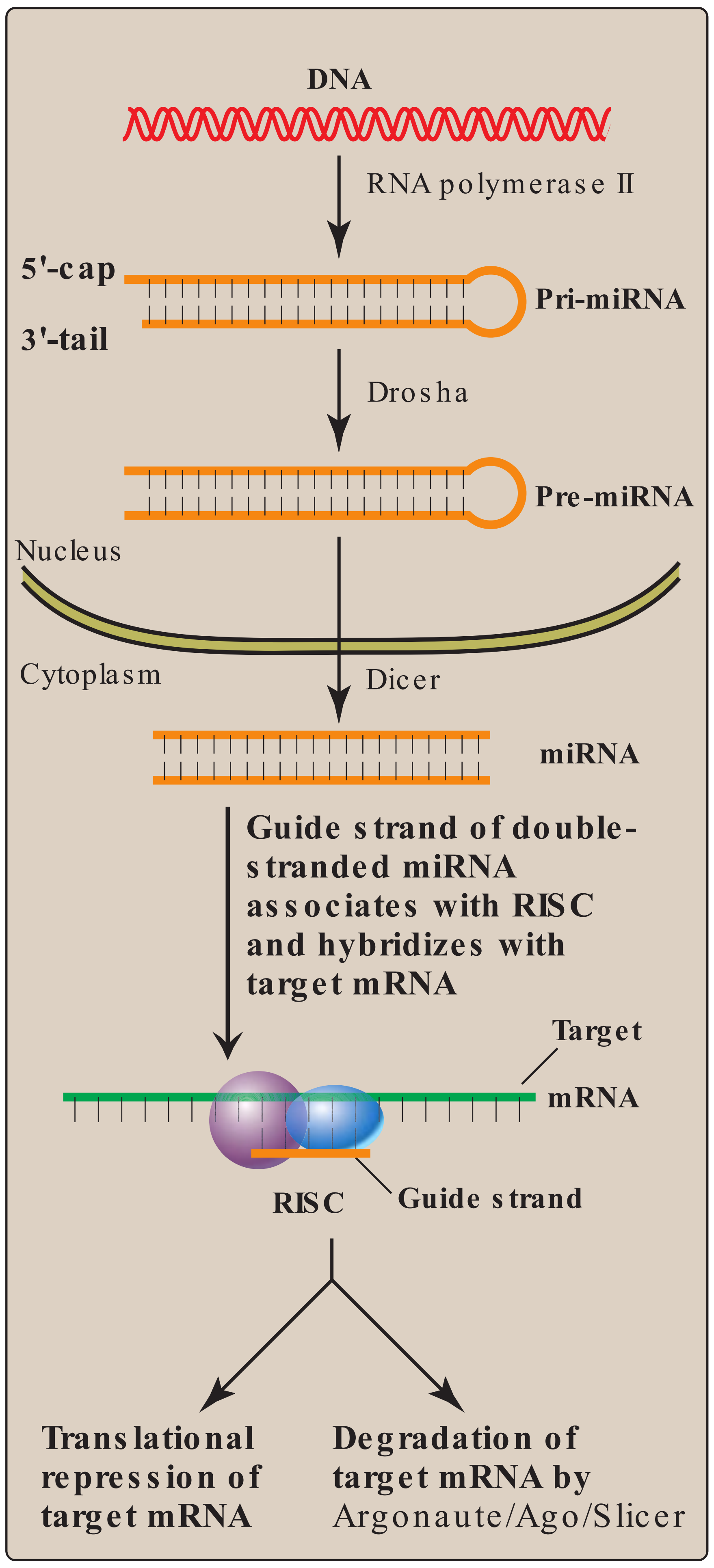

DNA
RNA polymerase II
5'-cap
3'-tail
Pri-miRNA
Drosha
Pre-miRNA
Nucleus
Cytoplasm
Dicer
miRNA
Guide strand of double-stranded miRNA associates with RISC and hybridizes with target mRNA
Target mRNA
RISC
Guide strand
Translational repression of target mRNA
Degradation of target mRNA by Argonaute/Ago/Slicer

Figure 32.15
Biogenesis and actions of miRNA. [Note: The extent of complementarity between the target messenger RNA (mRNA) and the microRNA (miRNA) determines the final outcome.] RISC = RNA-induced silencing complex.

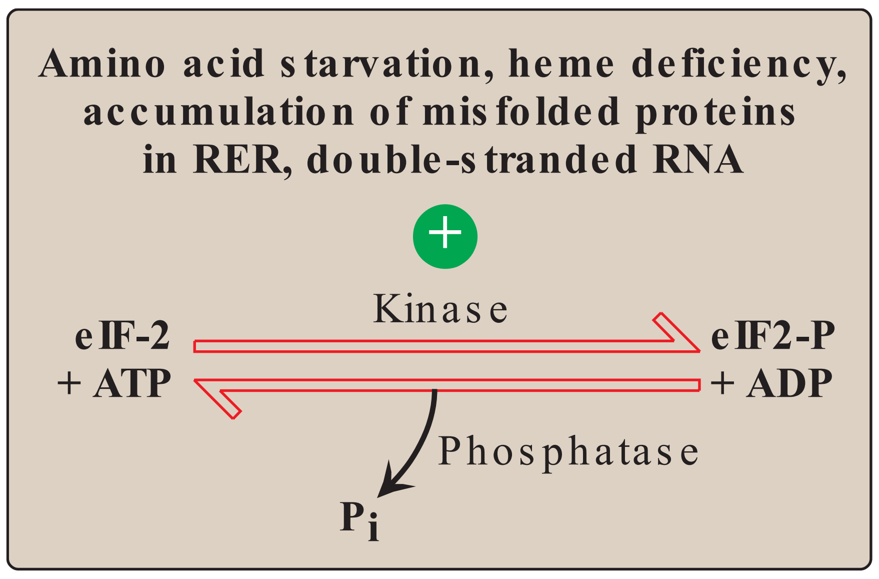

Amino acid starvation, heme deficiency, accumulation of misfolded proteins in RER, double-stranded RNA
+
Kinase
eIF-2
+ ATP
eIF2-P
+ ADP
Phosphatase
P_i

Figure 32.16
Regulation of translation initiation in eukaryotes by phosphorylation of eukaryotic translation initiation factor, eIF-2. RER = rough endoplasmic reticulum; ADP = adenosine diphosphate; P_i = inorganic phosphate.

translation initiation factor, eIF-2 (Figure 32.16). Phosphorylation of eIF-2 inhibits its function and so inhibits translation at the initiation step (see p. 443). [Note: Phosphorylation of eIF-2 prevents its reactivation by inhibiting GDP–GTP exchange.] Phosphorylation is catalyzed by kinases that are activated in response to environmental conditions, such as amino acid starvation, heme deficiency in erythroblasts, the presence of double-stranded RNA (signaling viral infection), and the accumulation of misfolded proteins in the rough endoplasmic reticulum.

D. Regulation through modifications to DNA

Gene expression in eukaryotes is also influenced by the availability of DNA to the transcriptional apparatus, the amount of DNA, and the arrangement of DNA. [Note: Localized transitions between the B and Z forms of DNA (see p. 398) can also affect gene expression.]

1. **Access to DNA:** In eukaryotes, DNA is found complexed with histone and nonhistone proteins to form chromatin (see p. 409). Transcriptionally active, decondensed chromatin (euchromatin) differs from the more condensed, inactive form (heterochromatin) in a number of ways. Active chromatin contains histone proteins that have been covalently modified at their amino terminal ends by acetylation or phosphorylation (see p. 422 for a discussion of histone acetylation/deacetylation by the histone acetyltransferase and histone deacetylase enzymes). Such modifications decrease the positive charge of these basic proteins, thereby decreasing the strength of their association with negatively charged DNA. This relaxes the nucleosome (see p. 409), allowing transcription factors access to specific regions on the DNA. Nucleosomes can also be repositioned, an ATP-requiring process called chromatin remodeling. Another difference between transcriptionally active and inactive chromatin is the extent of methylation of cytosine bases in CG-rich regions (CpG islands) in the promoter region of many genes. Methylation is by methyltransferases that use S-adenosylmethionine as the methyl donor (Figure 32.17). Transcriptionally active genes are less methylated (hypomethylated) than their inactive counterparts, suggesting that DNA hypermethylation silences gene expression. [Note: Modification of histones and methylation of DNA are epigenetic. They are heritable changes in DNA that alter gene expression without altering the base sequence.]

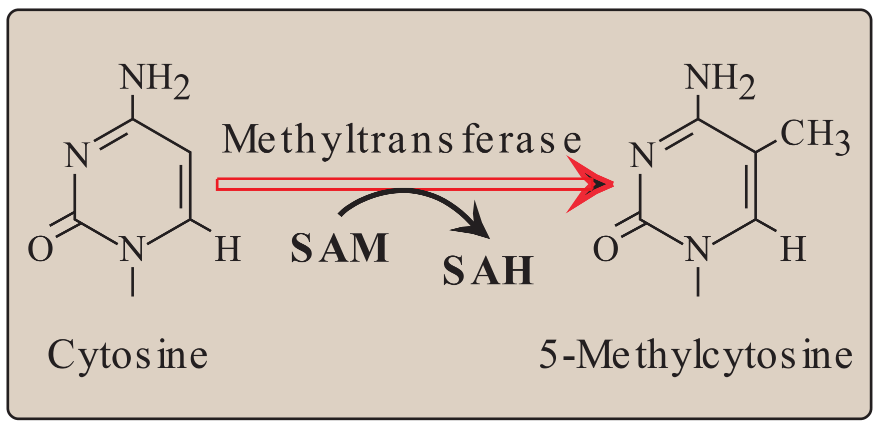


Figure 32.17
The methylation of cytosine in eukaryotic DNA. SAM = S-adenosylmethionine; SAH = S-adenosylhomocysteine.

2. **Amount of DNA:** A change up or down in the number of copies of a gene can affect the amount of gene product produced. An increase in copy number (gene amplification) has contributed to increased genomic complexity and is still a normal developmental process in certain nonmammalian species. In mammals, however, gene amplification is seen with some diseases and in response to particular chemotherapeutic drugs such as methotrexate, an inhibitor of the enzyme dihydrofolate reductase (DHFR), required for the synthesis of thymidine triphosphate (TTP) in the pyrimidine biosynthetic pathway (see p. 304). TTP is essential for DNA synthesis. Gene amplification results in an increase in the number of DHFR genes and resistance to the drug, allowing TTP to be made.

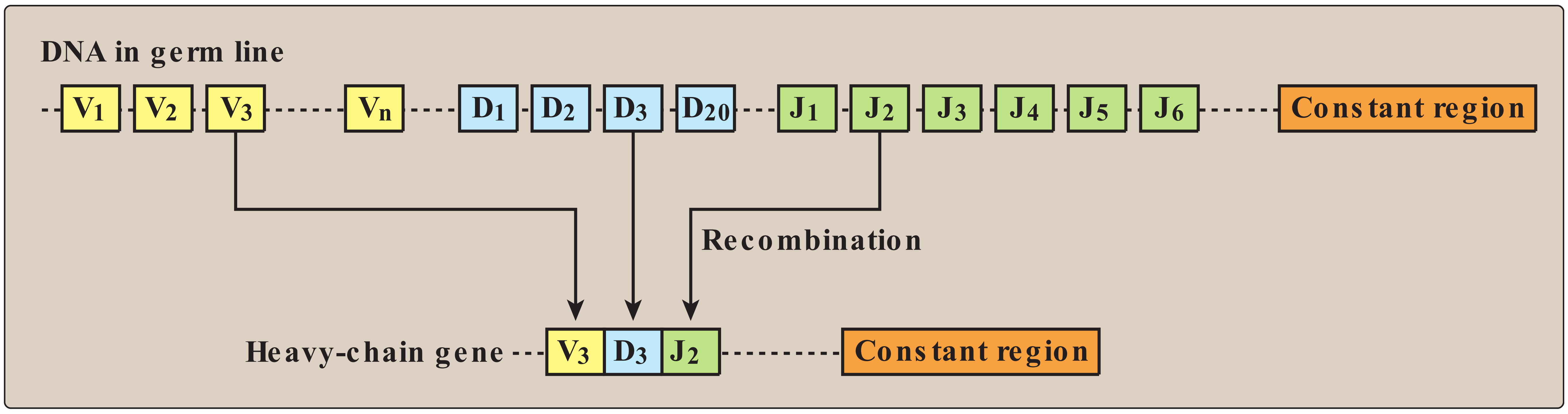


Figure 32.18
DNA rearrangements in the generation of immunoglobulins. V= variable; D = diversity; J = joining.

3. **Arrangement of DNA:** The process by which immunoglobulins (antibodies) are produced by B lymphocytes involves permanent rearrangements of the DNA in these cells. The immunoglobulins (for example, IgG) consist of two light and two heavy chains, with each chain containing regions of variable and constant amino acid sequence. The variable region is the result of somatic recombination of segments within both the light- and the heavy-chain genes. During B-lymphocyte development, single variable (V), diversity (D), and joining (J) gene segments are brought together through gene rearrangement to form a unique variable region (Figure 32.18). This process allows the generation of 10^9–10^{11} different immunoglobulins from a single gene, providing the diversity needed for the recognition of an enormous number of antigens. [Note: The shift from the membrane-bound form to the secreted form of immunoglobulins involves poly-A site choice (see p. 426).]

4. **Mobile DNA elements:** Transposons (Tns) are mobile segments of DNA that move in an essentially random manner from one site to another on the same or a different chromosome. Movement is mediated by transposase, an enzyme encoded by the Tn itself. Movement can be direct, in which transposase cuts out and then inserts the Tn at a new site, or replicative, in which the Tn is copied and the copy inserted elsewhere while the original remains in place. In eukaryotes, including humans, replicative transposition frequently involves an RNA intermediate, in which case the Tn is called a retrotransposon (see p. 408). Transposition has contributed to structural variation in the genome but also has the potential to alter gene expression and even to cause disease. Although the vast majority of retrotransposons in the human genome have lost the ability to move, some are still active. Their transposition is thought to be the basis for some rare cases of hemophilia A and Duchenne muscular dystrophy. [Note: The growing problem of antibiotic-resistant bacteria is a consequence, at least in part, of the exchange of plasmids among bacterial cells. If the plasmids contain Tns carrying antibiotic resistance genes, the recipient bacteria gain resistance to one or more antimicrobial drugs.]

V. CHAPTER SUMMARY

Gene expression results in the production of a functional gene product (either RNA or protein) through the processes of replication, transcription, and translation (Figure 32.19). **Genes** can be either **constitutive** (always expressed, housekeeping genes) or **regulated** (expressed only under certain conditions in all cells or in a subset of cells). The ability to appropriately **express** (**positive regulation**) or **repress** (**negative regulation**) genes is essential in all organisms. **Regulation** of gene expression occurs primarily at the level of **transcription** in both **prokaryotes** and **eukaryotes** and is mediated through the **binding** of **trans-acting proteins to cis-acting regulatory elements on the DNA**. In **eukaryotes**, regulation also occurs through **modifications to the DNA** as well as through **posttranscriptional** and **posttranslational events**. In **prokaryotes**, such as E. coli, the coordinate regulation of genes whose protein products are required for a particular process is achieved through **operons** (groups of genes sequentially arranged on the chromosome along with the regulatory elements that determine their transcription). The **lac operon** contains the Z, Y, and A structural genes, the protein products of which are needed for the catabolism of lactose. It is subject to negative and positive regulation. When **glucose is available**, the **operon is repressed** by the binding of the **repressor protein** (the product of the lacI gene) to the **operator**, thus preventing transcription. When **only lactose is present**, the **operon is induced** by an isomer of lactose (**allolactose**) that binds the repressor protein, preventing it from binding to the operator. In addition, **cyclic AMP binds the catabolite-activator protein (CAP)**, and the complex **binds the DNA** at the **CAP site**. This **increases promoter efficiency** and results in the expression of the structural genes through the production of a **polycistronic messenger RNA (mRNA)**. When both **glucose and lactose are present**, glucose prevents formation of cAMP and **transcription** of these genes is **negligible**. The **trp operon** contains genes needed for the synthesis of tryptophan (Trp), and, like the lac operon, it is regulated by **negative control**. Unlike the lac operon, it is **also regulated by attenuation**, in which mRNA synthesis that escaped repression by Trp is terminated before completion. **Transcription** of **ribosomal RNA** and **transfer RNA** is selectively **inhibited** in prokaryotes by the **stringent response to amino acid starvation**. **Translation** is also a site of **prokaryotic gene regulation**: Excess ribosomal proteins bind the Shine-Dalgarno sequence on their own polycistronic mRNA, preventing ribosomes from binding. **Gene regulation** is **more complex in eukaryotes**. **Operons** typically are **not present**, but **coordinate regulation** of the transcription of genes located on different chromosomes can be achieved through the **binding of trans-acting proteins to cis-acting elements**. In **multicellular organisms**, **hormones** can **cause coordinated regulation**, either through the **binding** of the **hormone receptor–hormone complex to the DNA** (as with steroid hormones) or through the **binding of a protein** that is activated in **response to a second messenger** (as with glucagon). In each case, binding to DNA is mediated through structural motifs such as the **zinc finger. Co- and posttranscriptional regulation** is also seen in **eukaryotes** and includes **splice-site choice**, **polyA-site choice**, **mRNA editing**, and variations in **mRNA stability** as seen with transferrin receptor synthesis and with **RNA interference**. **Regulation at the translational level** can be caused by the **phosphorylation and inhibition of eukaryotic initiation factor, eIF-2**. Gene expression in eukaryotes is also influenced by availability of DNA to the transcriptional apparatus, the amount of DNA, and the arrangement of the DNA. **Epigenetic changes** to histone proteins and DNA also influence gene expression.

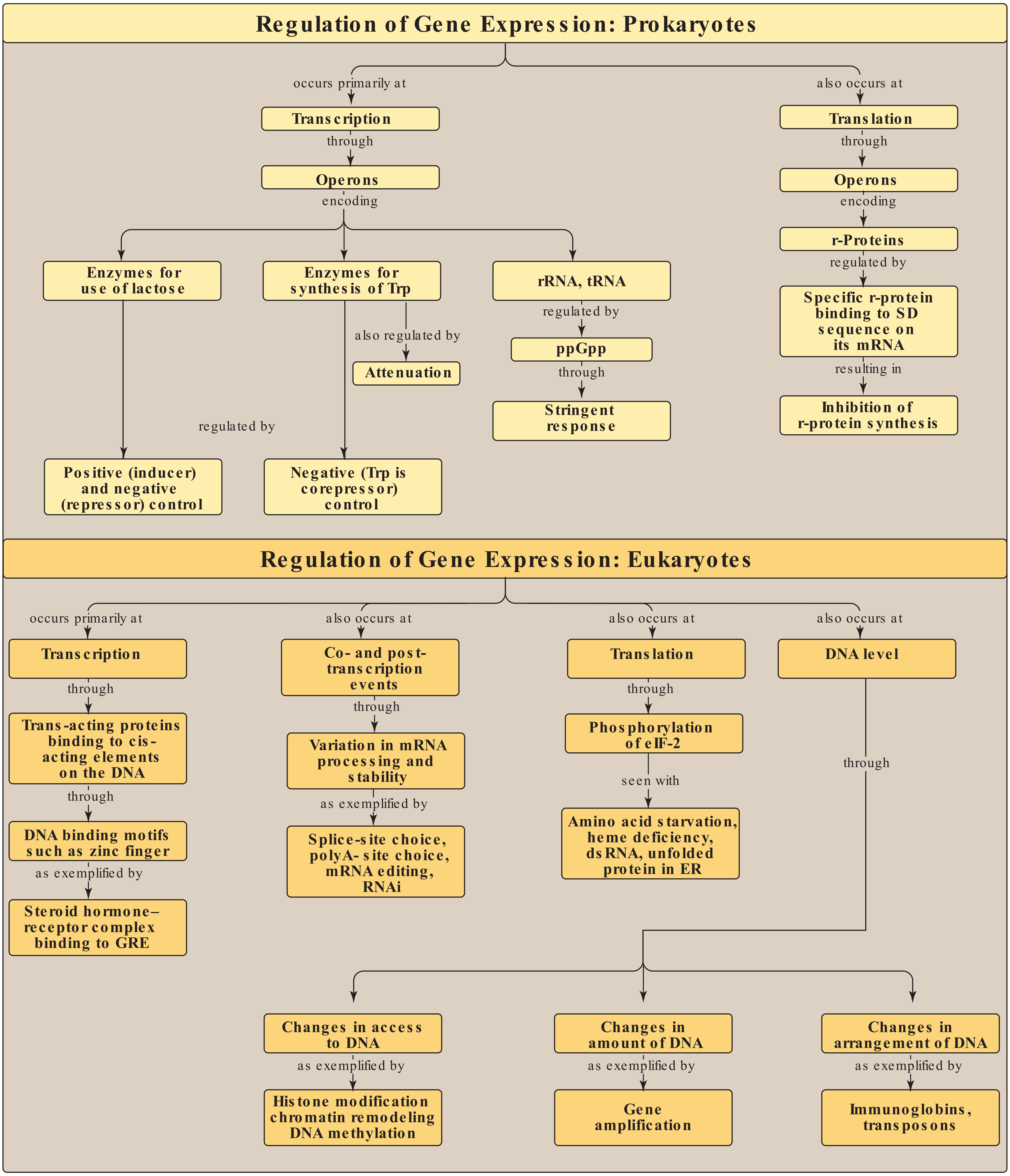

Regulation of Gene Expression: Prokaryotes
occurs primarily at
Transcription
through
Operons
encoding
Enzymes for use of lactose
Enzymes for synthesis of Trp
also regulated by
Attenuation
regulated by
Positive (inducer) and negative (repressor) control
Negative (Trp is corepressor) control
rRNA, tRNA
regulated by
ppGpp
through
Stringent response
also occurs at
Translation
through
Operons
encoding
r-Proteins
regulated by
Specific r-protein binding to SD sequence on its mRNA
resulting in
Inhibition of r-protein synthesis
Regulation of Gene Expression: Eukaryotes
occurs primarily at
Transcription
through
Trans-acting proteins binding to cis-acting elements on the DNA
through
DNA binding motifs such as zinc finger
as exemplified by
Steroid hormone–receptor complex binding to GRE
also occurs at
Co- and post-transcription events
through
Variation in mRNA processing and stability
as exemplified by
Splice-site choice, polyA-site choice, mRNA editing, RNAi
also occurs at
Translation
through
Phosphorylation of eIF-2
seen with
Amino acid starvation, heme deficiency, dsRNA, unfolded protein in ER
also occurs at
DNA level
through
Changes in access to DNA
as exemplified by
Histone modification chromatin remodeling DNA methylation
Changes in amount of DNA
as exemplified by
Gene amplification
Changes in arrangement of DNA
as exemplified by
Immunoglobins, transposons

Figure 32.19
Summary of key concepts for the regulation of gene expression. GRE = glucocorticoid-response element; ppGpp = polyphosphorylated guanosine; r-protein = ribosomal protein; SD sequence = Shine-Dalgarno sequence; RNAi = RNA interference; eIF-2 = eukaryotic initiation factor 2.

Biotechnology and Human Disease

33

I. OVERVIEW

In the past, efforts to understand genes and their expression have been confounded by the immense size and complexity of human deoxyribonucleic acid (DNA). The human genome contains approximately three billion (10^9) base pairs (bp) that encode 20,000 to 25,000 protein-coding genes located on 23 chromosomes in the haploid genome. It is now possible to determine the nucleotide sequence of long stretches of DNA, and the entire human genome has been sequenced. This effort (called the Human Genome Project and completed in 2003) was made possible by several techniques that have already contributed to our understanding of many genetic diseases (Figure 33.1). These include, first, the discovery of restriction endonucleases that permit the cleavage of huge DNA molecules into defined fragments. Second, the development of cloning techniques that provide a mechanism for amplification of specific nucleotide sequences. Finally, the ability to synthesize specific probes, which has allowed the identification and manipulation of nucleotide sequences of interest. These and other experimental approaches have permitted the identification of both normal and mutant nucleotide sequences in DNA. This knowledge has led to the development of methods for the diagnosis of genetic diseases and some successes in the treatment of patients by gene therapy. [Note: The genomes of several viruses, prokaryotes, and nonhuman eukaryotes have also been sequenced.]

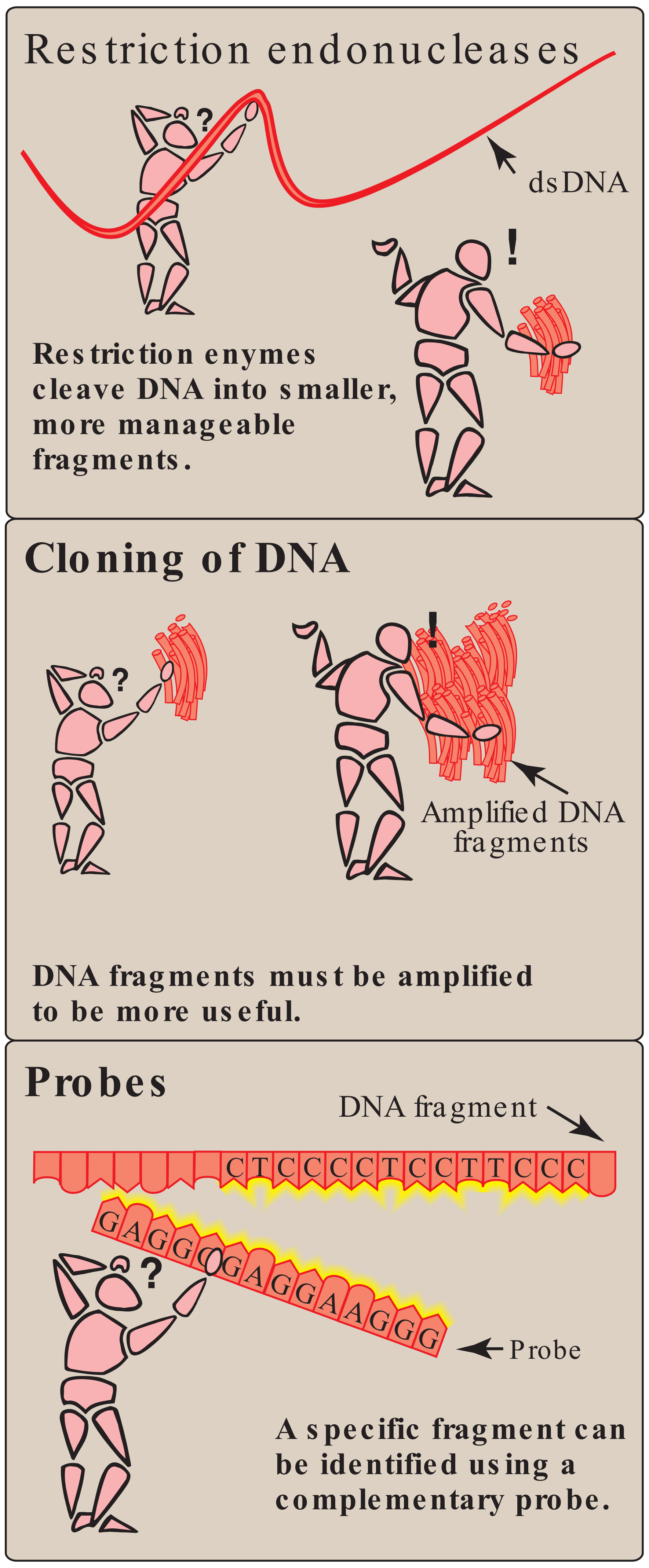

Restriction endonucleases
?
dsDNA
!
Restriction enymes cleave DNA into smaller, more manageable fragments.
Cloning of DNA
?
!
Amplified DNA fragments
DNA fragments must be amplified to be more useful.
Probes
DNA fragment
CTCCCCTCCTTCCC
GAGGGGAGGAAGGG
?
Probe
A specific fragment can be identified using a complementary probe.

Figure 33.1
Three techniques that facilitate analysis of human DNA. dsDNA = double-stranded DNA.

II. RESTRICTION ENDONUCLEASES

One of the major obstacles to molecular analysis of genomic DNA is the immense size of the molecules involved. The discovery of a special group of bacterial enzymes, called restriction endonucleases (restriction enzymes), which cleave double-stranded (ds) DNA into smaller, more manageable fragments, opened the way for DNA analysis. Because each enzyme cleaves DNA at a specific nucleotide sequence (restriction site), restriction enzymes are used experimentally to obtain precisely defined DNA segments called restriction fragments.

A. Specificity of restriction endonucleases

Restriction endonucleases recognize short stretches of dsDNA (four to eight bp) that contain specific nucleotide sequences. These

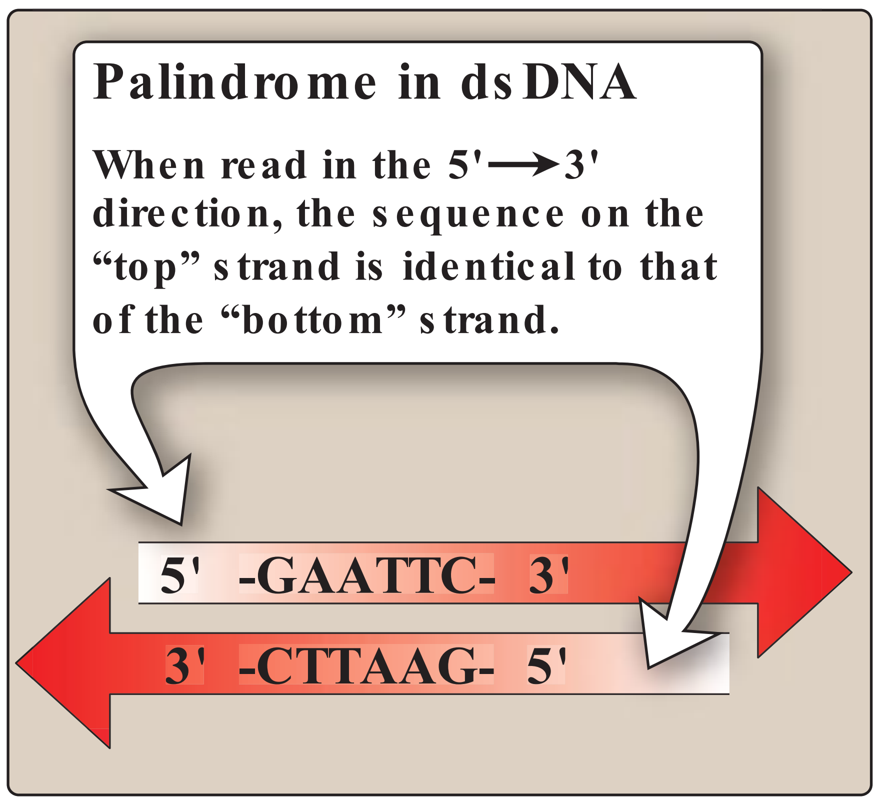

Palindrome in dsDNA
When read in the 5'⟶3' direction, the sequence on the "top" strand is identical to that of the "bottom" strand.
5' -GAATTC- 3'
3' -CTTAAG- 5'

Figure 33.2
Recognition sequence of restriction endonuclease EcoRI shows twofold rotational symmetry. dsDNA = double-stranded DNA; A = adenine; C = cytosine; G = guanine; T = thymine.

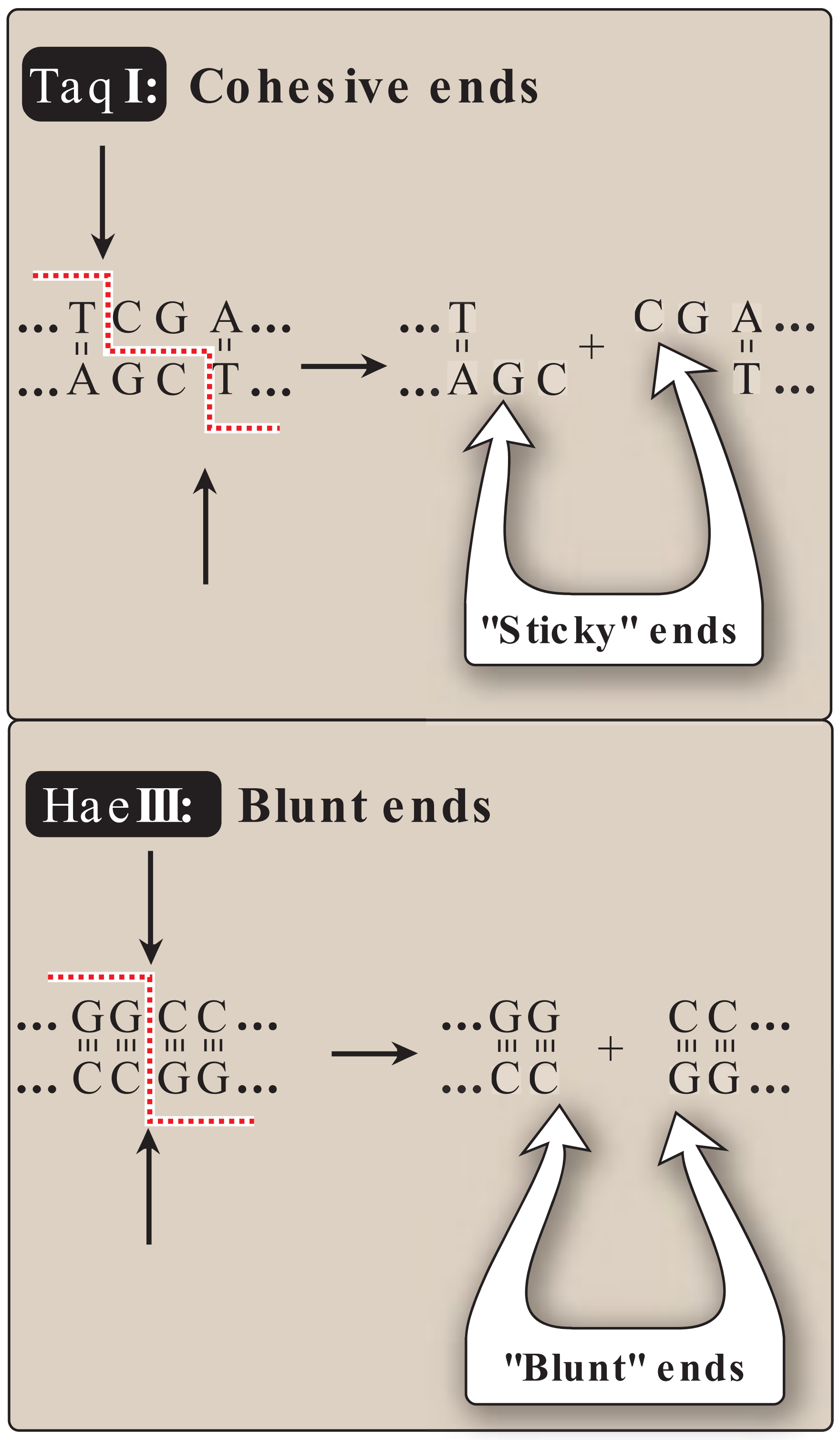


Figure 33.3
Specificity of TaqI and HaeIII restriction endonucleases; A = adenine; C = cytosine; G = guanine; T = thymine.

sequences, which differ for each restriction enzyme, are palindromes, that is, they exhibit twofold rotational symmetry (Figure 33.2). This means that, within a short region of the double helix, the nucleotide sequence on the two strands is identical if each is read in the 5'→3' direction. Therefore, if you turn the page upside down (that is, rotate it 180° around its axis of symmetry) the sequence remains the same.

In bacteria, restriction endonucleases "restrict" the expression of nonbacterial (foreign) DNA through cleavage. Bacterial DNA is protected by methylation of bases at the restriction site.

B. Nomenclature

A restriction enzyme is named according to the organism from which it was isolated. The first letter of the name is from the genus of the bacterium. The next two letters are from the name of the species. An additional letter indicates the type or strain, and a number (Roman numeral) is appended to indicate the order in which the enzyme was discovered in that particular organism. For example, HaeIII is the third restriction endonuclease isolated from the bacterium Haemophilus aegyptius.

C. "Sticky" and "blunt" ends

Restriction enzymes cleave dsDNA so as to produce a 3'-hydroxyl group on one end and a 5'-phosphate group on the other. Some restriction endonucleases, such as TaqI, form staggered cuts that produce "sticky" or cohesive ends (that is, the resulting DNA fragments have single-stranded sequences that are complementary to each other) as shown in Figure 33.3. Other restriction endonucleases, such as HaeIII, produce fragments that have "blunt" ends that are double stranded and, therefore, do not form hydrogen bonds with each other. Using the enzyme DNA ligase (see p. 406), sticky ends of a DNA fragment of interest can be covalently joined with other DNA fragments that have sticky ends produced by cleavage with the same restriction endonuclease (Figure 33.4). [Note: A ligase encoded by bacteriophage T4 can covalently join blunt-ended fragments.]

D. Restriction sites

A DNA sequence that is recognized and cut by a restriction enzyme is called a restriction site. Restriction endonucleases cleave dsDNA into fragments of different sizes depending upon the size of the sequence recognized. For example, an enzyme that recognizes a specific 4-bp sequence produces many cuts in the DNA molecule, one every 4^4 bp. In contrast, an enzyme requiring a unique sequence of 6 bp produces fewer cuts (one every 4^6 bp) and, therefore, longer pieces. Hundreds of these enzymes, each having different cleavage specificities (varying in both nucleotide sequences and length of recognition sites), are commercially available.

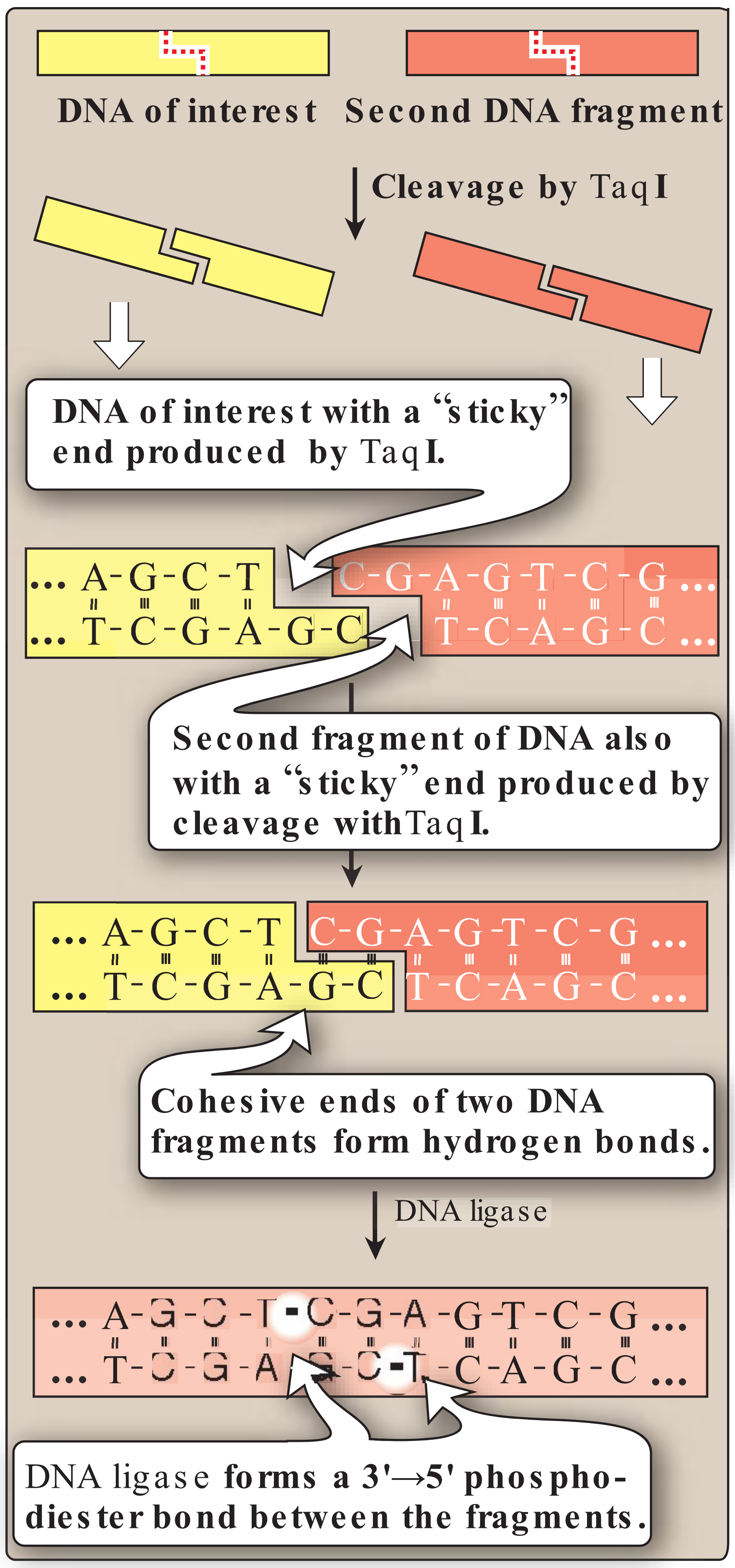
III. DNA CLONING

Introduction of a foreign DNA molecule into a replicating cell permits the cloning or amplification (that is, the production of many identical copies) of that DNA. [Note: Human DNA for cloning can be obtained from blood, saliva, and solid tissue.] In some cases, a single DNA fragment can be isolated and purified prior to cloning. More commonly, to clone a nucleotide sequence of interest, the total cellular DNA is first cleaved with a specific restriction enzyme, creating hundreds of thousands of fragments. Each of the resulting DNA fragments is joined to a DNA vector molecule (referred to as a cloning vector) to form a hybrid, or recombinant, DNA molecule. Each recombinant molecule carries its inserted DNA fragment into a single host cell (for example, a bacterium), where it is replicated. [Note: The process of introducing foreign DNA into a cell is called transformation for bacteria and yeast and transfection for higher eukaryotes.] As the host cell multiplies, it forms a clone in which every bacterium contains copies of the same inserted DNA fragment, hence the name "cloning." The cloned DNA can be released from its vector by cleavage (using the appropriate restriction endonuclease) and isolated. By this mechanism, many identical copies of the DNA of interest can be produced. [Note: An alternative to amplification by biologic cloning, the polymerase chain reaction (PCR), is described on p. 479.]

A. Vectors

A vector is a molecule of DNA to which the fragment of DNA to be cloned is joined. Essential properties of a vector include: 1) it must be capable of autonomous replication within a host cell, 2) it must contain at least one specific nucleotide sequence recognized by a restriction endonuclease, and 3) it must carry at least one gene that confers the ability to select for the vector such as an antibiotic resistance gene. Commonly used vectors include plasmids and viruses.

1. **Prokaryotic plasmids:** Prokaryotic organisms typically contain single, large, circular chromosomes. In addition, most species of bacteria also normally contain small, circular, extrachromosomal DNA molecules called plasmids (Figure 33.5). Plasmid DNA undergoes replication that may or may not be synchronized to chromosomal division. Plasmids may carry genes that convey antibiotic resistance to the host bacterium and may facilitate the transfer of genetic information from one bacterium to another. They can be readily isolated from bacterial cells, their circular DNA cleaved at specific sites by restriction endonucleases, and up to 10 kb (kilobases) of foreign DNA (cut with the same restriction enzyme) inserted. The recombinant plasmid can be introduced into a bacterium, producing large numbers of copies of the plasmid. The bacteria are grown in the presence of antibiotics, thus selecting for cells containing the hybrid plasmids, which provide antibiotic resistance (Figure 33.6). Artificial plasmids are routinely constructed. An example is pRB322 (Figure 33.5), which contains an origin of replication, two antibiotic resistance genes, and over 40 unique restriction sites. Use of plasmids is limited by the size of the DNA that can be inserted.



DNA of interest with a "sticky" end produced by TaqI.

Second fragment of DNA also with a "sticky" end produced by cleavage with TaqI.

Cohesive ends of two DNA fragments form hydrogen bonds.

DNA ligase forms a 3'→5' phosphodiester bond between the fragments.

Figure 33.4
Formation of recombinant DNA from restriction fragments with "sticky" ends. A = adenine; C = cytosine; G = guanine; T = thymine.

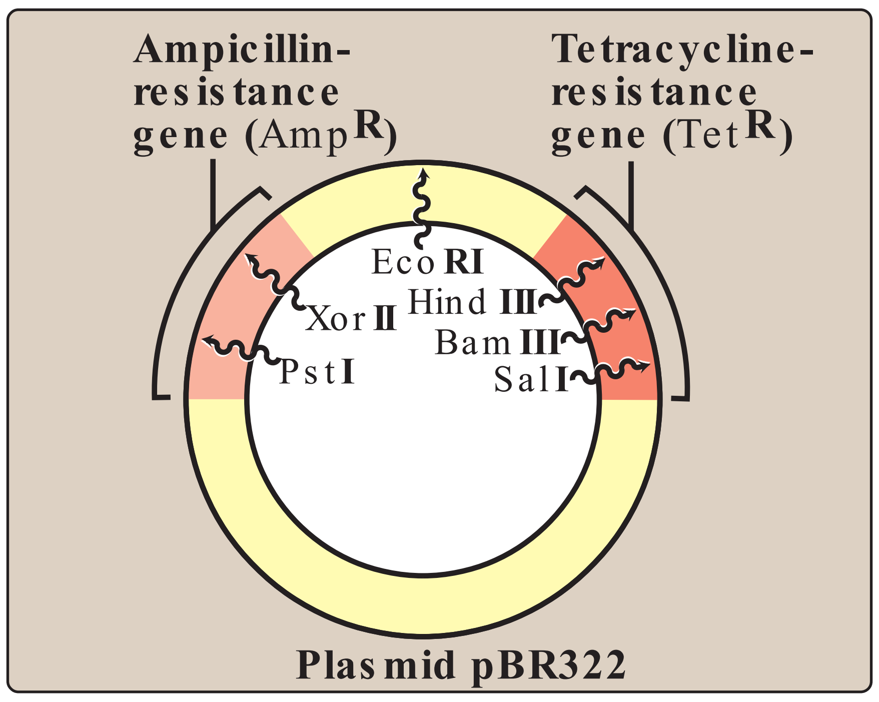
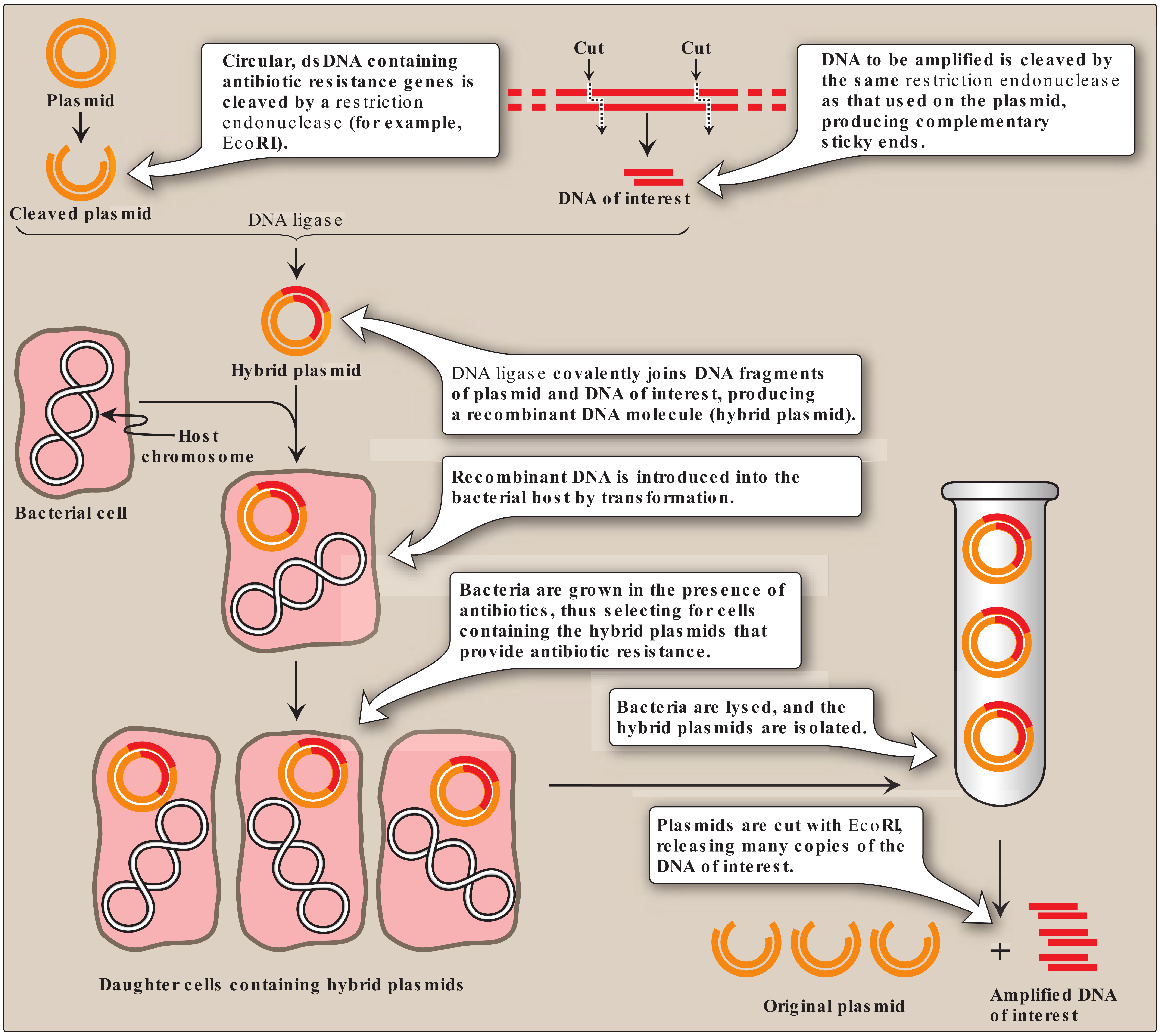

Ampicillin-resistance gene (Amp^R)
Tetracycline-resistance gene (Tet^R)
Eco RI
Hind III
Bam III
Sal I
Xor II
Pst I
Plasmid pBR322

Figure 33.5
A restriction map of plasmid pBR322 indicating the positions of its antibiotic resistance genes and 6 of the over 40 unique sites recognized by specific restriction endonucleases.


Plasmid
Cleaved plasmid
Cut
Cut
DNA of interest
DNA ligase
Hybrid plasmid
Host chromosome
Bacterial cell
Daughter cells containing hybrid plasmids
Original plasmid
+
Amplified DNA of interest

Circular, dsDNA containing antibiotic resistance genes is cleaved by a restriction endonuclease (for example, EcoRI).

DNA to be amplified is cleaved by the same restriction endonuclease as that used on the plasmid, producing complementary sticky ends.

DNA ligase covalently joins DNA fragments of plasmid and DNA of interest, producing a recombinant DNA molecule (hybrid plasmid).

Recombinant DNA is introduced into the bacterial host by transformation.

Bacteria are grown in the presence of antibiotics, thus selecting for cells containing the hybrid plasmids that provide antibiotic resistance.

Bacteria are lysed, and the hybrid plasmids are isolated.

Plasmids are cut with EcoRI, releasing many copies of the DNA of interest.

Figure 33.6
Summary of gene cloning. dsDNA = double-stranded DNA.

2. **Other vectors:** The development of improved vectors that can more efficiently accommodate larger DNA segments, or express the passenger genes in different cell types, has aided molecular genetics research. In addition to the prokaryotic plasmids described above, naturally occurring viruses that infect bacteria (bacteriophage 1, for example) or mammalian cells (retroviruses, for example), as well as artificial constructs such as cosmids and bacterial or yeast artificial chromosomes (BACs or YACs, respectively), are currently used as cloning vectors. [Note: BACs and YACs can accept DNA inserts of 100–250 kb and 250–1000 kb, respectively.]

B. DNA libraries

A DNA library is a collection of cloned restriction fragments of the DNA of an organism. Two kinds of libraries are commonly used: genomic libraries and complementary DNA (cDNA) libraries. Genomic libraries ideally contain a copy of every DNA nucleotide sequence in the genome. In contrast, cDNA libraries contain those DNA sequences that only appear as processed messenger RNA (mRNA) molecules, and these differ from one cell type to another. [Note: cDNA lacks introns and the control regions of the genes, whereas these are present in genomic DNA.]

1. **Genomic DNA libraries:** A genomic library is created by digestion of the total DNA of an organism with a restriction endonuclease and subsequent ligation to an appropriate vector. The recombinant DNA molecules replicate within host bacteria. Thus, the amplified DNA fragments collectively represent the entire genome of the organism and are called a genomic library. Regardless of the restriction enzyme used, the chances are rather good that the gene of interest contains more than one restriction site recognized by that enzyme. If this is the case, and if the digestion is allowed to go to completion, the gene of interest is fragmented (that is, it is not contained in any one clone in the library). To avoid this usually undesirable result, a partial digestion is performed in which either the amount or the time of action of the enzyme is limited. This results in cleavage occurring at only a fraction of the restriction sites on any one DNA molecule, thus producing fragments of about 20 kb. Enzymes that cut very frequently (that is, those that recognize four-bp sequences) are generally used for this purpose so that the result is an almost random collection of fragments. This ensures a high degree of probability that the gene of interest is contained, intact, in some fragment.

2. **Complementary DNA libraries:** If a protein-coding gene of interest is expressed at a high level in a particular tissue, the mRNA transcribed from that gene is likely also present at high concentrations in the cells of that tissue. For example, reticulocyte mRNA is composed largely of molecules encoding the a-globin and b-globin chains of hemoglobin. This mRNA can be used as a template to make a cDNA molecule using the enzyme reverse transcriptase (Figure 33.7). The resulting cDNA is, therefore, a double-stranded copy of mRNA. [Note: The template mRNA is isolated from transfer RNA and ribosomal RNA by the presence of its poly-A tail.] cDNA can be amplified by cloning or by PCR. It can be used as a probe to locate the gene that coded for the original mRNA (or fragments of the gene) in mixtures containing many unrelated DNA fragments. If the mRNA used as a template is a mixture of many different size species, the resulting cDNA is heterogeneous. These mixtures can be cloned to form a cDNA library. Because cDNA has no intervening sequences, it can be cloned into an expression vector for the synthesis of eukaryotic proteins by bacteria (Figure 33.8). These special plasmids contain a bacterial promoter for transcription of the cDNA and a Shine-Dalgarno (SD) sequence (see p. 438) that allows the bacterial ribosome to initiate translation of

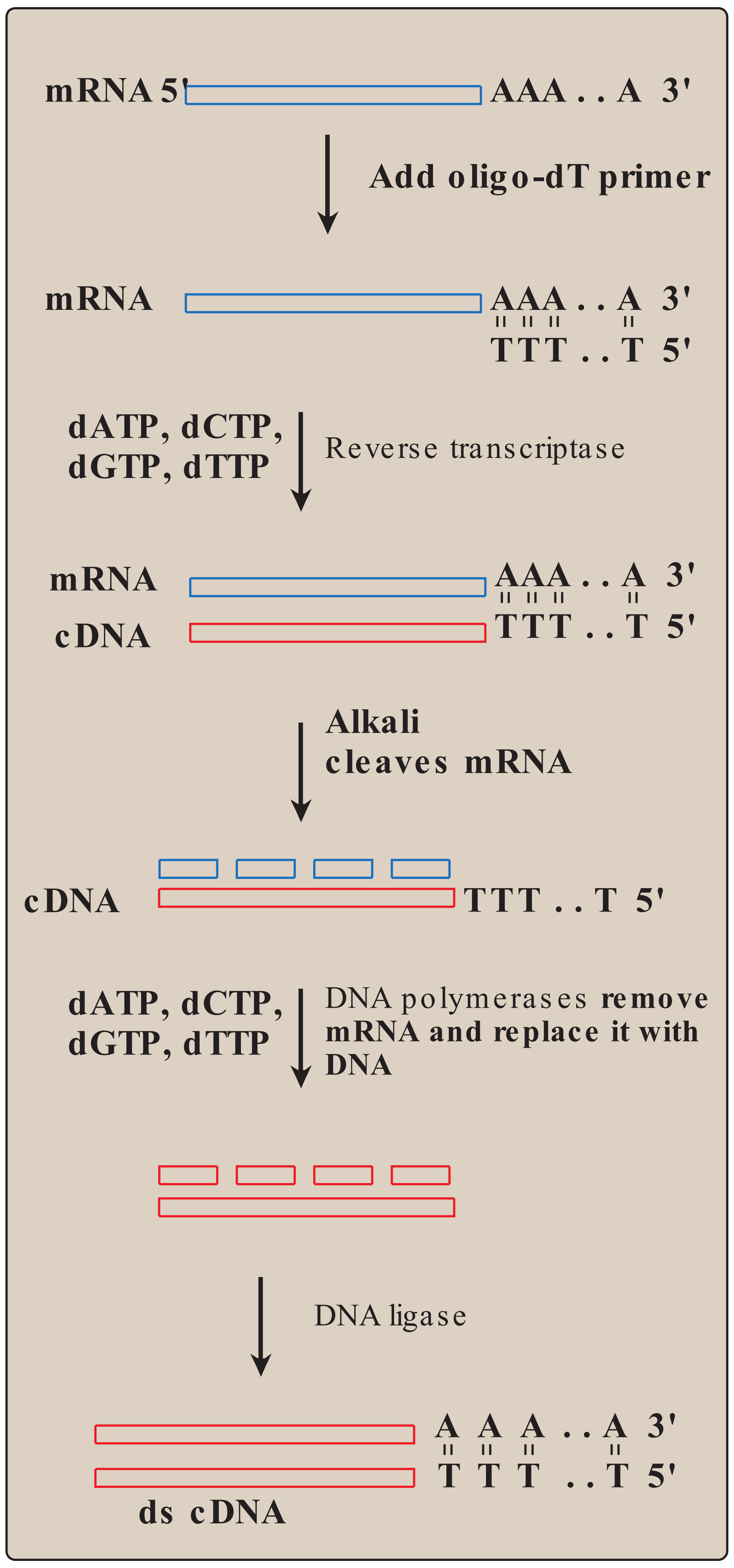


Figure 33.7
Synthesis of cDNA from messenger RNA (mRNA) using reverse transcriptase. Additional steps (not shown) are required to clone the cDNA. [Note: Recall that DNA is resistant to alkaline hydrolysis.]

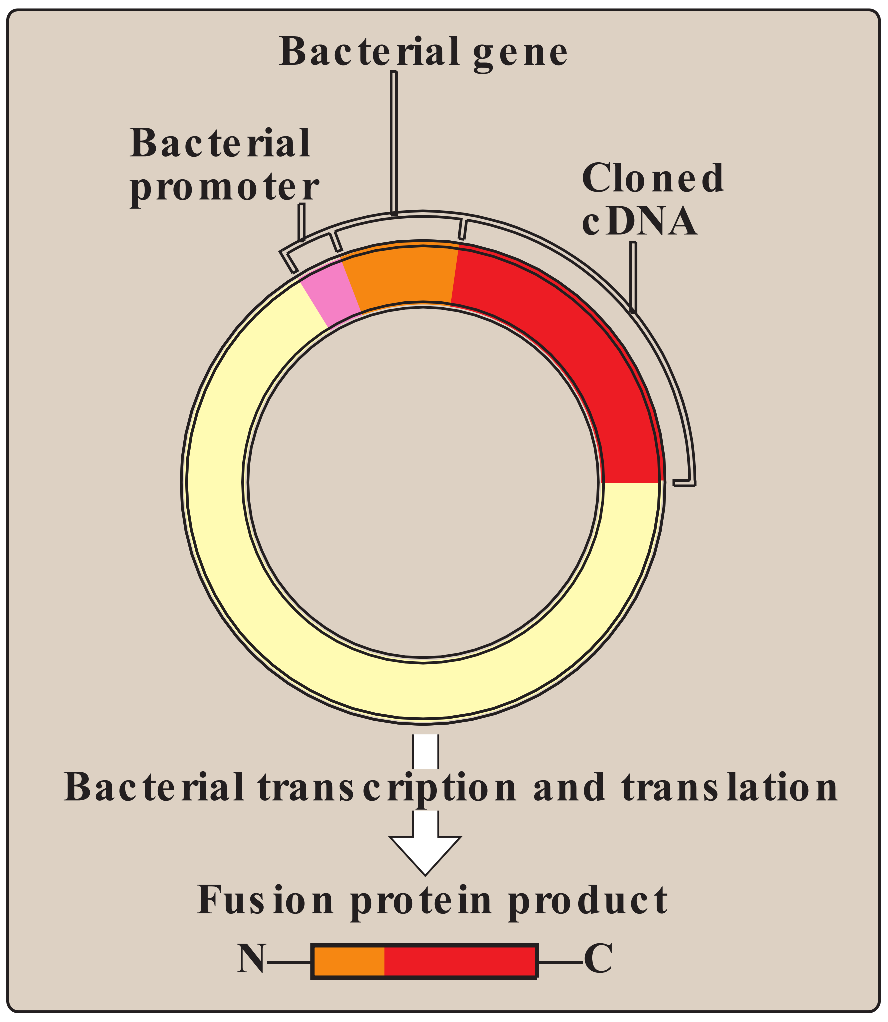


Figure 33.8
An expression vector. The complementary DNA (cDNA) is inserted within a bacterial gene, downstream of the promoter sequence, and the sequences for the messenger RNA Shine-Dalgarno sequence, start codon, and codons for the first few amino acids of the bacterial protein. The product is a fusion protein that contains just some amino acids of the bacterial protein 🟧 and all the amino acids of the cDNA-encoded protein 🟥.

the resulting mRNA molecule. The cDNA is inserted downstream of the promoter and within a gene for a protein that is expressed in the bacterium (for example, lacZ; see p.450), such that the mRNA produced contains an SD sequence, a few codons for the bacterial protein, and all the codons for the eukaryotic protein. This allows for more efficient expression and results in the production of a fusion protein. [Note: Therapeutic human insulin is made in bacteria through this technology. However, the extensive co- and posttranslational modifications (see p. 443) required for most other human proteins (for example, blood clotting factors) necessitates the use of eukaryotic, even mammalian, hosts.]

C. Sequencing of cloned DNA fragments

The base sequence of DNA fragments that have been cloned can be determined. The original procedure for this purpose was the Sanger dideoxy method illustrated in Figure 33.9. In this method, the single-stranded DNA (ssDNA) to be sequenced is used as the template for DNA synthesis by DNA polymerase. A radiolabeled primer complementary to the 3'-end of the target DNA is added, along with the four deoxyribonucleoside triphosphates (dNTPs). The sample is divided into four reaction tubes, and a small amount of one of the four dideoxyribonucleoside triphosphates (ddNTPs) is added to each tube. Because it contains no 3'-hydroxyl group, incorporation of a ddNMP terminates elongation at that point. The products of this reaction, then, consist of a mixture of DNA strands of different lengths, each terminating at a specific base. Separation of the various DNA products by size using polyacrylamide gel electrophoresis, followed by autoradiography, yields a pattern of bands from which the DNA base sequence can be read. [Note: The shorter the fragment, the farther it travels on the gel, with the shortest fragment representing that which was made first (that is, the 5'-end).] In place of a labeled primer, a mixture of the four ddNTPs linked to different fluorescent dyes and in a single reaction tube is now commonly used. [Note: The Human Genome Project used highly automated variations of this technique to determine the base sequence of the human genome.] Advances in sequencing technology, so-called next generation, or deep sequencing, now allow the sequencing of longer segments in a shorter time with increased fidelity and decreased cost through the simultaneous (parallel) sequencing of many DNA pieces.

IV. PROBES

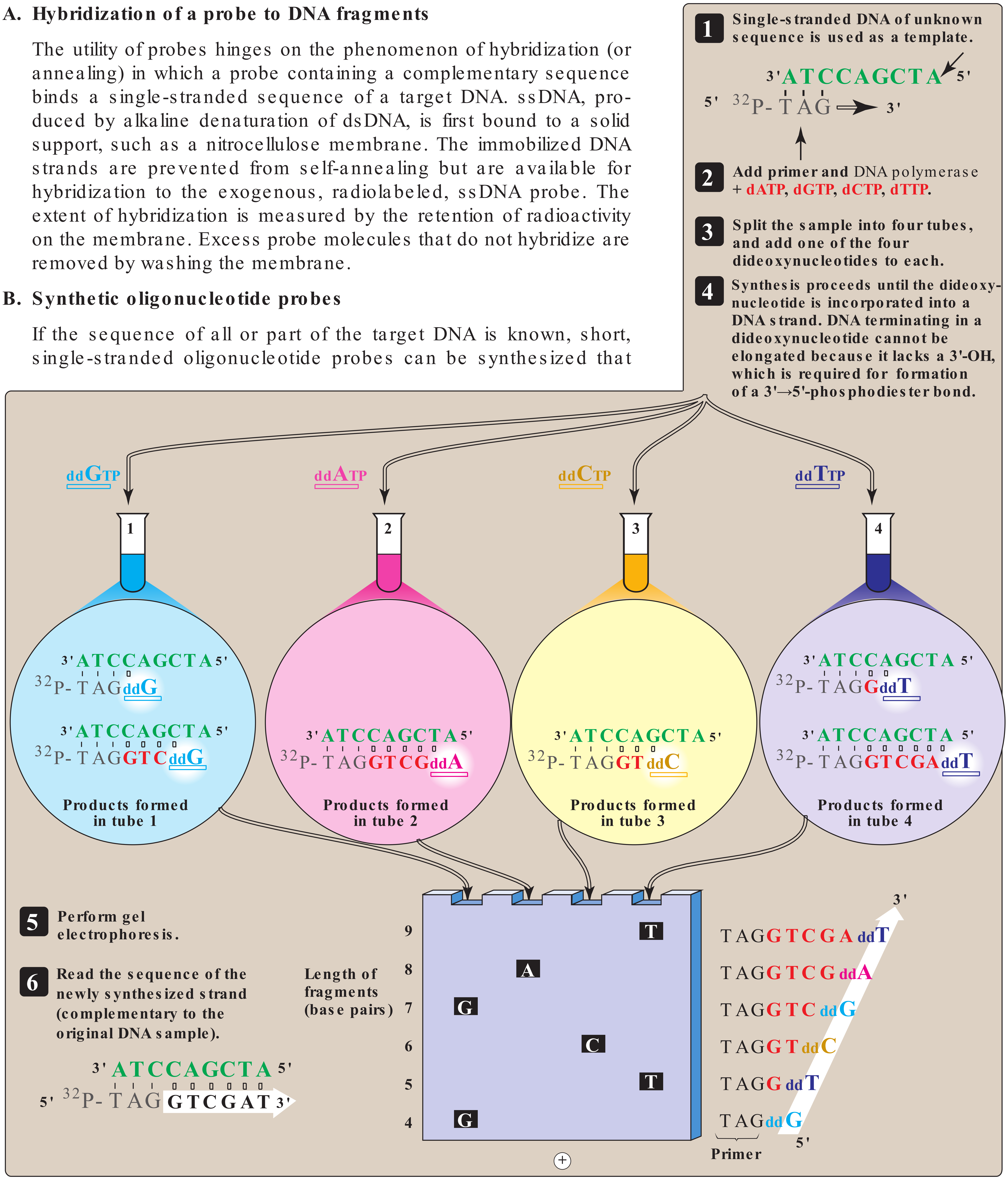
Cleavage of large DNA molecules by restriction enzymes produces a bewildering array of fragments. How can the DNA sequence of interest be picked out of a mixture of thousands or even millions of irrelevant DNA fragments? The answer lies in the use of a probe, a short piece of ssDNA or RNA, labeled with a radioisotope, such as ^{32}P, or with a nonradioactive molecule, such as biotin or a fluorescent dye. The sequence of a probe is complementary to a sequence in the DNA of interest, called the target DNA. Probes are used to identify which band on a gel or which clone in a library contains the target DNA, a process called screening.

A. Hybridization of a probe to DNA fragments

The utility of probes hinges on the phenomenon of hybridization (or annealing) in which a probe containing a complementary sequence binds a single-stranded sequence of a target DNA. ssDNA, produced by alkaline denaturation of dsDNA, is first bound to a solid support, such as a nitrocellulose membrane. The immobilized DNA strands are prevented from self-annealing but are available for hybridization to the exogenous, radiolabeled, ssDNA probe. The extent of hybridization is measured by the retention of radioactivity on the membrane. Excess probe molecules that do not hybridize are removed by washing the membrane.

B. Synthetic oligonucleotide probes

If the sequence of all or part of the target DNA is known, short, single-stranded oligonucleotide probes can be synthesized that



1 Single-stranded DNA of unknown sequence is used as a template.

2 Add primer and DNA polymerase + dATP, dGTP, dCTP, dTTP.

3 Split the sample into four tubes, and add one of the four dideoxynucleotides to each.

4 Synthesis proceeds until the dideoxynucleotide is incorporated into a DNA strand. DNA terminating in a dideoxynucleotide cannot be elongated because it lacks a 3'-OH, which is required for formation of a 3'→5'-phosphodiester bond.

5 Perform gel electrophoresis.

6 Read the sequence of the newly synthesized strand (complementary to the original DNA sample).

3' ATCCAGCTA 5'
5' 32P- TAG 3'
ddGTP
ddATP
ddCTP
ddTTP
1
2
3
4
3' ATCCAGCTA 5'
32P- TAGddG
3' ATCCAGCTA 5'
32P- TAGGTCddG
Products formed in tube 1
3' ATCCAGCTA 5'
32P- TAGGTCGddA
Products formed in tube 2
3' ATCCAGCTA 5'
32P- TAGGTddC
Products formed in tube 3
3' ATCCAGCTA 5'
32P- TAGGddT
3' ATCCAGCTA 5'
32P- TAGGTCGAddT
Products formed in tube 4
Length of fragments (base pairs)
9
8
7
6
5
4
T
A
G
C
T
G
+
3'
TAGGTCGAddT
TAGGTCGddA
TAGGTCddG
TAGGTddC
TAGGddT
TAGddG
5'
Primer
3' ATCCAGCTA 5'
5' 32P- TAG GTCGAT 3'

Figure 33.9
DNA sequencing by the Sanger dideoxy method. [Note: The original method utilized a radiolabeled primer. Fluorescent dye-labeled ddNTPs are now commonly used.] A = adenine; C = cytosine; G = guanine; T = thymine; d = deoxy; dd = dideoxy.

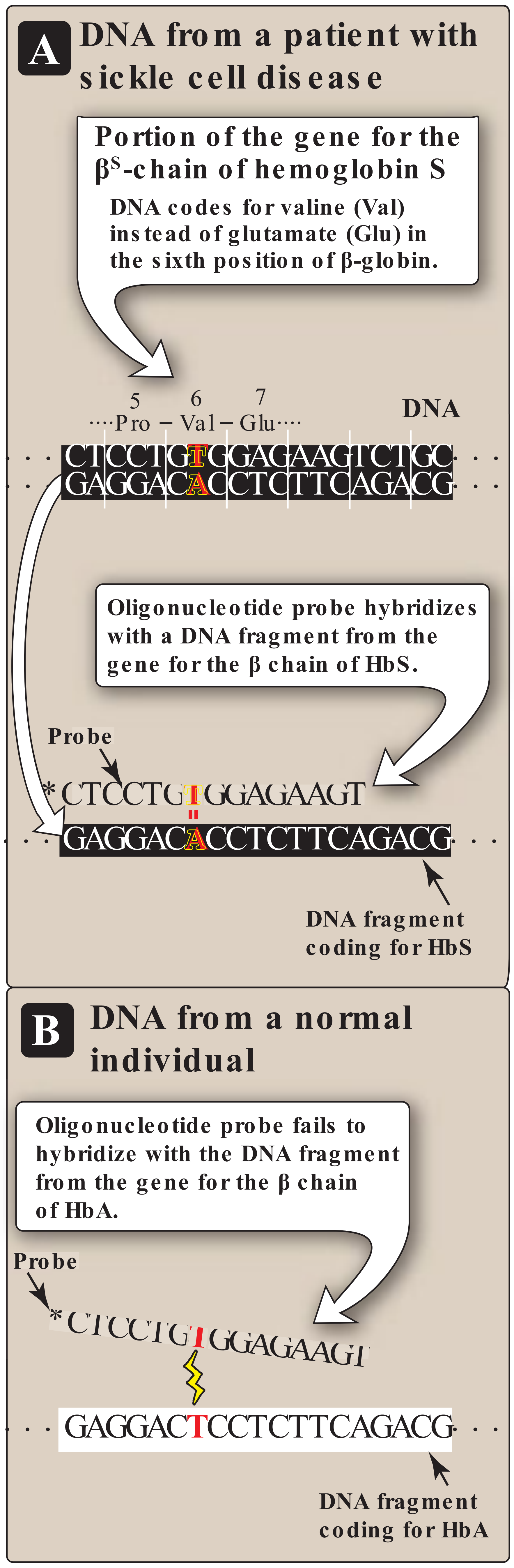

A
DNA from a patient with sickle cell disease
Portion of the gene for the β^S-chain of hemoglobin S
DNA codes for valine (Val) instead of glutamate (Glu) in the sixth position of β-globin.
5 6 7
····Pro – Val – Glu····
DNA
CTCCTGTGGAGAAGTCTGC
GAGGACACCTCTTCAGACG
Oligonucleotide probe hybridizes with a DNA fragment from the gene for the β chain of HbS.
Probe
*CTCCTGTGGAGAAGT
GAGGACACCTCTTCAGACG
DNA fragment coding for HbS
B
DNA from a normal individual
Oligonucleotide probe fails to hybridize with the DNA fragment from the gene for the β chain of HbA.
Probe
*CTCCTGTGGAGAAGT
GAGGACTCCTCTTCAGACG
DNA fragment coding for HbA

Figure 33.10
Allele-specific oligonucleotide probe detects hemoglobin (Hb) S allele. [Note: * indicates ^{32}P radiolabel.] A = adenine; C = cytosine; G = guanine; T = thymine; Pro = proline.

are complementary to a small region of the gene of interest. If the sequence of the gene is unknown, the amino acid sequence of the protein, the final gene product, may be used to construct a nucleic acid probe using the genetic code as a guide. Because of the degeneracy of the genetic code, it is necessary to synthesize several oligonucleotides. [Note: Oligonucleotides can be used to detect single-base changes in the sequence to which they are complementary. In contrast, cDNA probes contain many thousands of bases, and their binding to a target DNA with a single-base change is unaffected.]

1. **Detecting the b^S-globin mutation:** A synthetic allele-specific oligonucleotide (ASO) probe can be used to detect the presence of the sickle cell mutation in the b-globin gene (Figure 33.10). DNA, isolated from leukocytes and amplified, is denatured and applied to a membrane. A radiolabeled oligonucleotide probe, complementary to the point mutation (GAG → GTG, glutamate → valine) at codon 6 in patients with the b^S gene, is applied to the membrane. DNA isolated from a heterozygous individual (sickle cell trait) or a homozygous patient (sickle cell disease) contains a sequence that is complementary to the probe, and a double-stranded hybrid forms that can be detected by electrophoresis. In contrast, DNA obtained from normal individuals is not complementary at this positon and, therefore, does not form a hybrid (see Figure 33.10). Use of a pair of such ASO probes (one specific for the normal allele and one specific for the mutant allele) allows all three possible genotypes (homozygous normal, heterozygous, and homozygous mutant) to be distinguished (Figure 33.11). [Note: ASO probes are useful only if the mutation and its location are known.]

C. Biotinylated probes

Because the disposal of radioactive waste is becoming increasingly expensive, nonradiolabeled probes have been developed. One of the most successful is based on the vitamin biotin (see p. 381), which can be chemically linked to the nucleotides used to synthesize the probe. Biotin was chosen because it binds very tenaciously to avidin, a readily available protein contained in chicken egg whites. Avidin can be attached to a fluorescent dye detectable optically with great sensitivity. Thus, a DNA fragment (displayed, for example, by gel electrophoresis) that hybridizes with the biotinylated probe can be made visible by immersing the gel in a solution of dye-coupled avidin. After washing away the excess avidin, the DNA fragment that binds the probe is fluorescent. [Note: Labeled probes can allow detection and localization of DNA or RNA sequences in cell or tissue preparations, a process called in situ hybridization (ISH). If the probe is fluorescent, the technique is called FISH.]

D. Antibodies

If no amino acid sequence information is available to guide the synthesis of a probe for direct detection of the DNA of interest, a gene can be identified indirectly by cloning cDNA in an expression vector that allows the cloned cDNA to be transcribed and translated. A labeled antibody is used to identify which bacterial colony produces the protein and, therefore, contains the cDNA of interest.

V. SOUTHERN BLOTTING

Southern blotting is a technique that combines the use of restriction enzymes, electrophoresis, and DNA probes to generate, separate, and detect pieces of DNA.

A. Experimental procedure

This method, named after its inventor, Edward Southern, involves the following steps (Figure 33.12). First, DNA is extracted from cells, for example, a patient's leukocytes. Second, the DNA is cleaved into many fragments using a restriction enzyme. Third, the resulting fragments are separated on the basis of size by electrophoresis. [Note: Because the large fragments move more slowly than the smaller ones, the lengths of the fragments, usually expressed as the number of base pairs, can be calculated from comparison of the position of the band relative to standard fragments of known size.] The DNA fragments in the gel are denatured and transferred (blotted) to a nitrocellulose membrane for analysis. If the original DNA represents the individual's entire genome, the enzymic digest contains a million or more fragments. The gene of interest is on only one (or a few if the gene itself was fragmented) of these pieces of DNA. If all the DNA segments were visualized by a nonspecific technique, they would appear as an unresolved blur of overlapping bands. To avoid this, the last step in Southern blotting uses a probe to identify the DNA fragments of interest. The patterns observed on Southern blot analysis depend both on the specific restriction endonuclease and on the probe used to visualize the restriction fragments. [Note: Variants of the Southern blot have been facetiously named "Northern" (electrophoresis of mRNA followed by hybridization with a specific probe), and "Western" (electrophoresis of protein followed by detection with an antibody directed against the protein of interest), neither of which relates to anyone's name or to points of the compass.]

B. Detection of mutations

Southern blotting can detect DNA mutations such as large insertions, deletions, or rearrangements of nucleotides. It can also detect point mutations (replacement of one nucleotide by another; see p. 433) that cause the loss or gain of restriction sites. Such mutations cause the pattern of bands to differ from those seen with a normal gene. Longer fragments are generated if a restriction site is lost. For example, in Figure 33.12, Person 2 lacks a restriction site present in Person 1. Alternatively, the point mutation may create a new cleavage site with the production of shorter fragments. [Note: Most sequence differences at restriction sites are harmless variations in the DNA.]

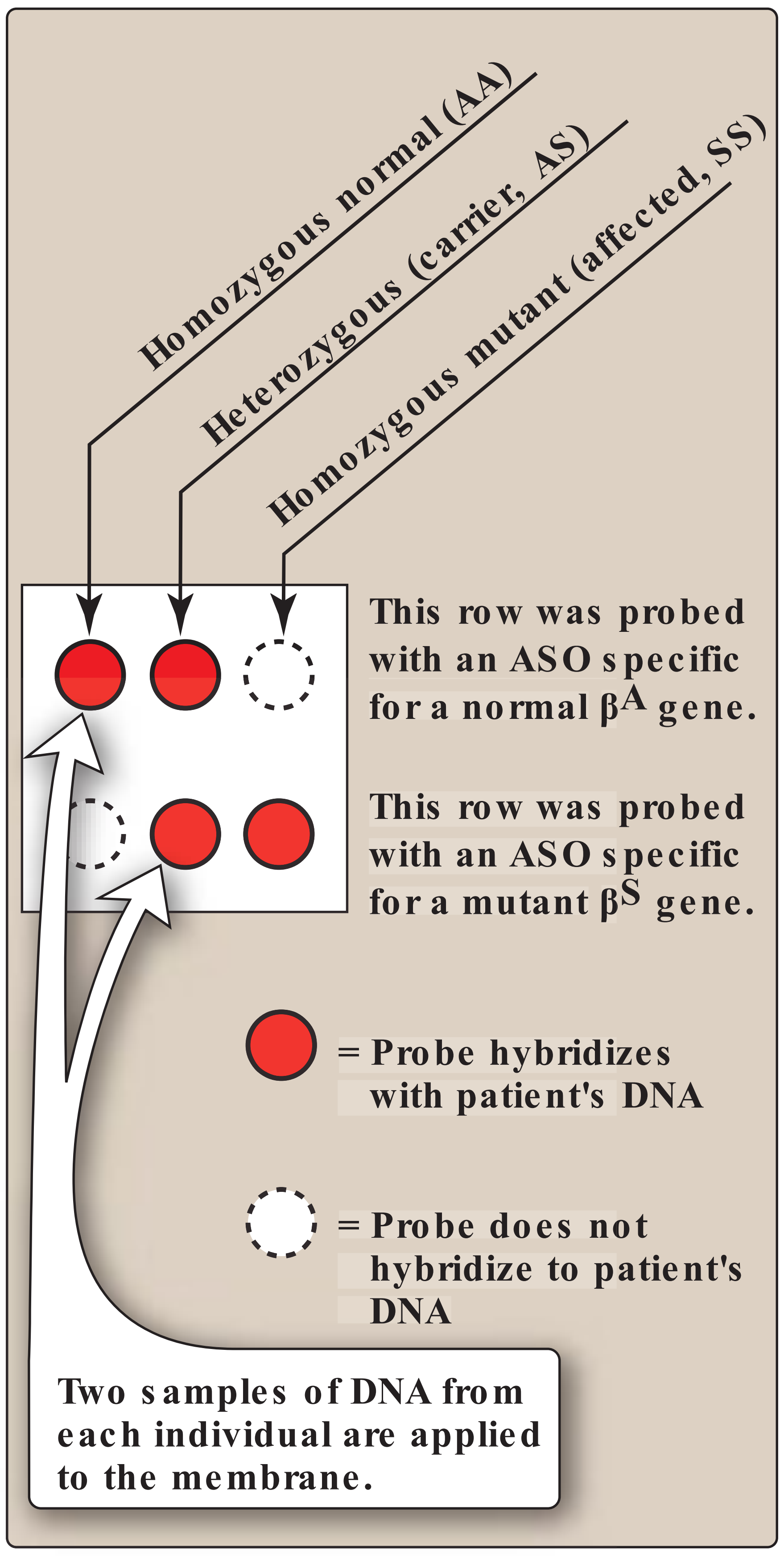
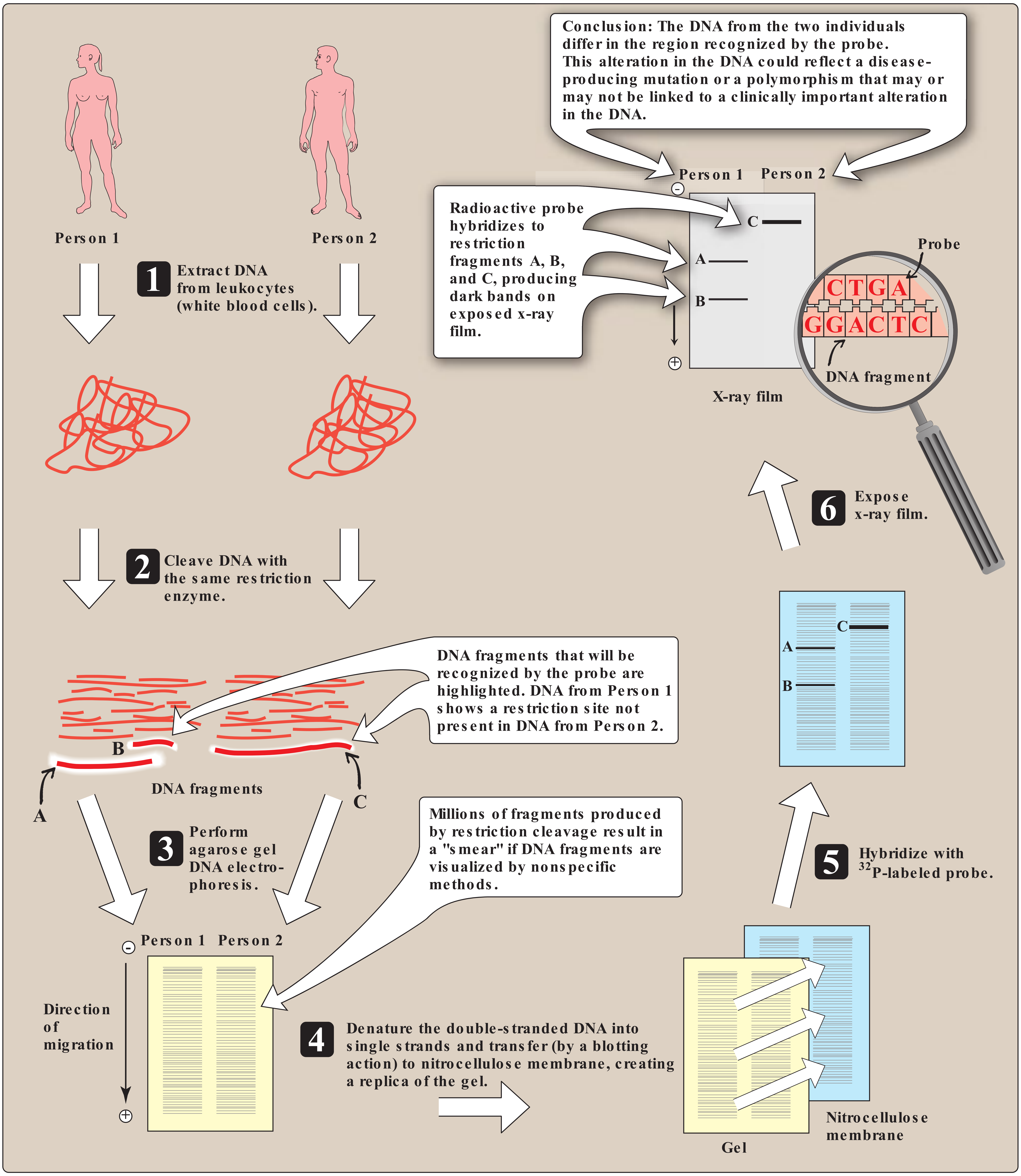

Homozygous normal (AA)
Heterozygous (carrier, AS)
Homozygous mutant (affected, SS)
This row was probed with an ASO specific for a normal β^A gene.
This row was probed with an ASO specific for a mutant β^S gene.
= Probe hybridizes with patient's DNA
= Probe does not hybridize to patient's DNA
Two samples of DNA from each individual are applied to the membrane.

Figure 33.11
Allele-specific oligonucleotide (ASO) probes used to detect the sickle cell mutation and differentiate between sickle cell trait and disease.

VI. RESTRICTION FRAGMENT LENGTH POLYMORPHISM

It has been estimated that the genomes of any two unrelated people are 99.5% identical. With 6 billion bp in the diploid human genome, that represents variation in about 30 million bp. These genome variations are the result of mutations that lead to polymorphisms. A polymorphism is a change in genotype that can result in no change in phenotype or a change in phenotype that is harmless; causes increased susceptibility



Person 1
Person 2
1
Extract DNA from leukocytes (white blood cells).
2
Cleave DNA with the same restriction enzyme.
B
A
C
DNA fragments
3
Perform agarose gel DNA electrophoresis.
-
Person 1
Person 2
Direction of migration
+
4
Denature the double-stranded DNA into single strands and transfer (by a blotting action) to nitrocellulose membrane, creating a replica of the gel.
Gel
Nitrocellulose membrane
5
Hybridize with ^{32}P-labeled probe.
A
B
C
6
Expose x-ray film.
Person 1
Person 2
-
C
A
B
+
X-ray film
Probe
CTGA
GGACTC
DNA fragment

DNA fragments that will be recognized by the probe are highlighted. DNA from Person 1 shows a restriction site not present in DNA from Person 2.

Millions of fragments produced by restriction cleavage result in a "smear" if DNA fragments are visualized by nonspecific methods.

Radioactive probe hybridizes to restriction fragments A, B, and C, producing dark bands on exposed x-ray film.

Conclusion: The DNA from the two individuals differ in the region recognized by the probe. This alteration in the DNA could reflect a disease-producing mutation or a polymorphism that may or may not be linked to a clinically important alteration in the DNA.

Figure 33.12
Southern blotting procedure. [Note: Nonradiolabeled probes are now commonly used.]

to a disease; or, rarely, causes the disease. It is traditionally defined as a sequence variation at a given locus (allele) in more than 1% of a population. Polymorphisms primarily occur in the 98% of the genome that does not encode proteins (that is, in introns and intergenic regions). A restriction fragment length polymorphism (RFLP) is a genetic variant that can be observed by cleaving the DNA into fragments (restriction fragments) with a restriction enzyme. The length of the restriction fragments is altered if the variant alters the DNA so as to create or abolish a site of restriction endonuclease cleavage (a restriction site). RFLP can be used to detect human genetic variations, for example, in prospective parents or in fetal tissue.

A. DNA variations resulting in restriction fragment length polymorphism

Two types of DNA variation commonly result in RFLP: single-base changes in the DNA sequence and tandem repeats of DNA sequences.

1. **Single-base changes in DNA:** About 90% of human genome variation comes in the form of single nucleotide polymorphisms (SNPs, pronounced "snips"), that is, variations that involve just one base (Figure 33.13). The substitution of one nucleotide at a restriction site can render the site unrecognizable by a particular restriction endonuclease. A new restriction site can also be created by the same mechanism. In either case, cleavage with an endonuclease results in fragments of lengths differing from the normal that can be detected by DNA hybridization (see Figure 33.12). The altered restriction site can be either at the site of a disease-causing mutation (rare) or at a site some distance from the mutation. [Note: The HapMap, developed by The International Haplotype Map Project, is a catalog of common SNPs in the human genome. The data are being used in genome-wide association studies (GWAS) to identify those alleles that affect health and disease.]

2. **Tandem repeats:** Polymorphism in chromosomal DNA can also arise from the presence of a variable number of tandem repeats [VNTR] see Figure 33.14). These are short sequences of DNA at scattered locations in the genome, repeated in tandem (one after another). The number of these repeat units varies from person to person but is unique for any given individual and, therefore, serves as a molecular fingerprint. Cleavage by restriction enzymes yields fragments that vary in length depending on how many repeated segments are contained in the fragment (Figure 33.14). Many different VNTR loci have been identified and are extremely useful for DNA fingerprint analysis, such as in forensic and paternity cases. It is important to emphasize that these polymorphisms, whether SNP or VNTR, are simply markers, which, in most cases, have no known effect on the structure, function, or rate of production of any particular protein.

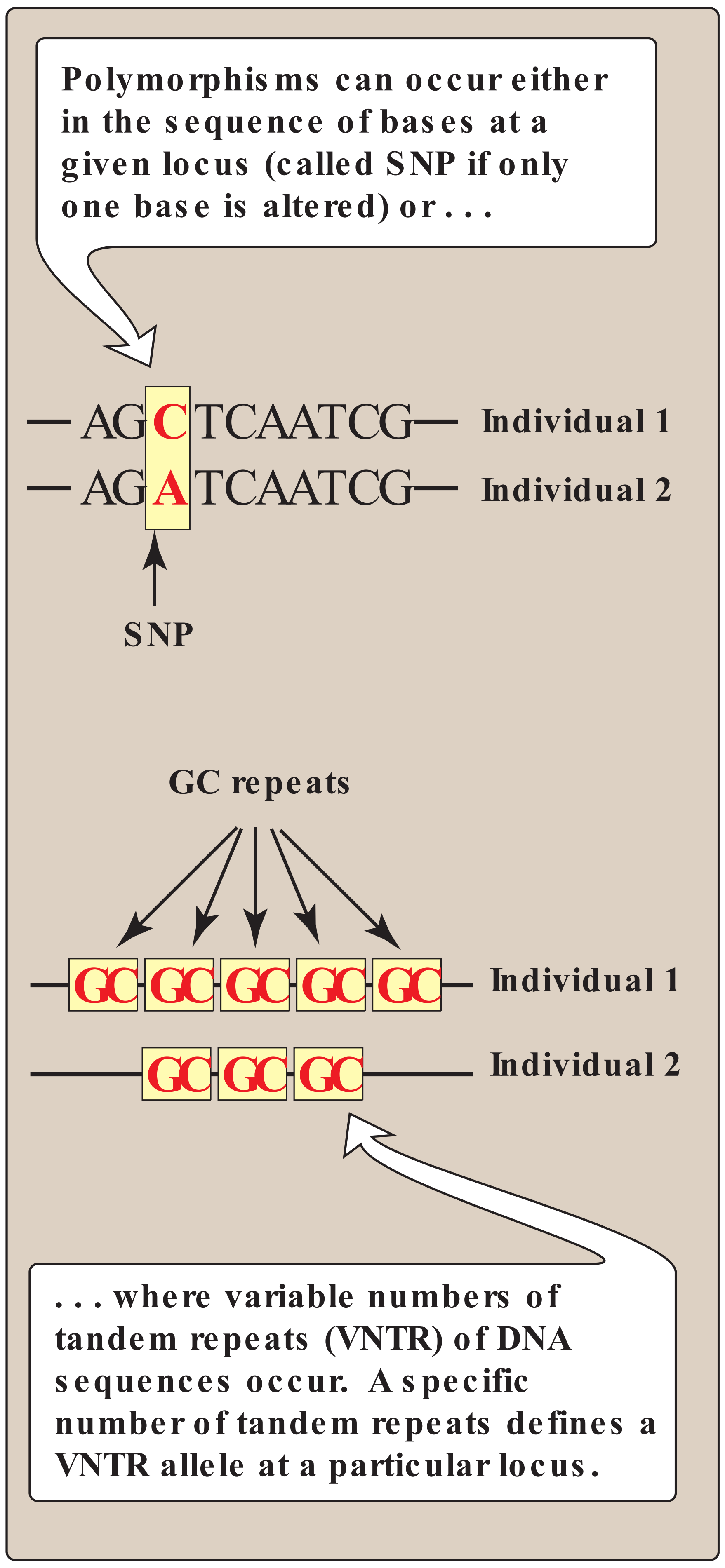
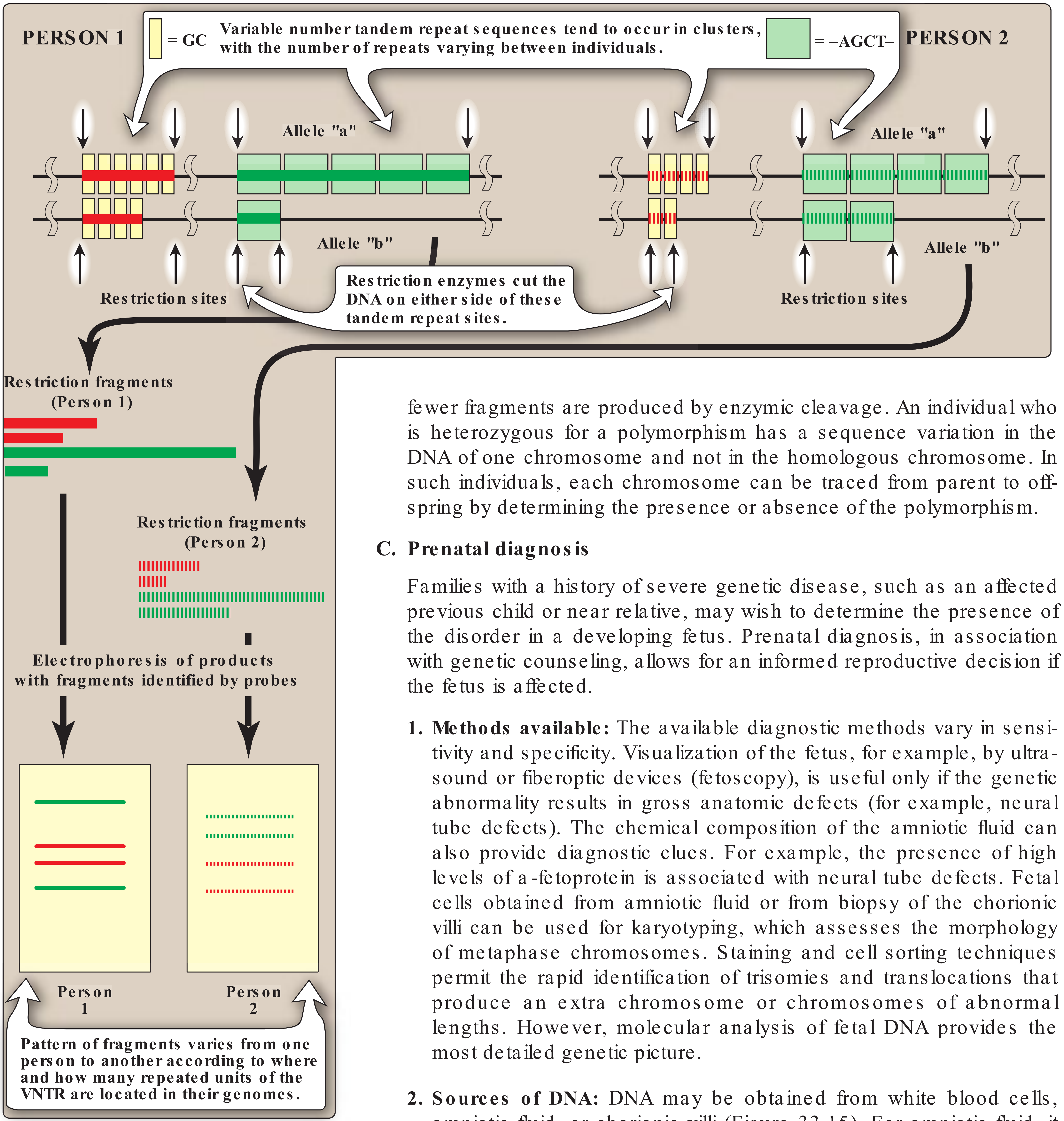

Polymorphisms can occur either in the sequence of bases at a given locus (called SNP if only one base is altered) or . . .
— AGCTCAATCG— Individual 1
— AGATCAATCG— Individual 2
SNP
GC repeats
GC GC GC GC GC Individual 1
GC GC GC Individual 2
. . . where variable numbers of tandem repeats (VNTR) of DNA sequences occur. A specific number of tandem repeats defines a VNTR allele at a particular locus.

Figure 33.13
Common forms of genetic polymorphism. SNP = single-nucleotide polymorphism. A = adenine; C = cytosine; G = guanine; T = thymine.

B. Tracing chromosomes from parent to offspring

If the DNA of an individual has gained a restriction site by base substitution, then enzymic cleavage yields at least one additional fragment. Conversely, if a mutation results in loss of a restriction site,


PERSON 1
= GC
= –AGCT–
PERSON 2
Allele "a"
Allele "b"
Restriction sites
Allele "a"
Allele "b"
Restriction sites
Restriction fragments (Person 1)
Restriction fragments (Person 2)
Electrophoresis of products with fragments identified by probes
Person 1
Person 2

Variable number tandem repeat sequences tend to occur in clusters, with the number of repeats varying between individuals.

Restriction enzymes cut the DNA on either side of these tandem repeat sites.

Pattern of fragments varies from one person to another according to where and how many repeated units of the VNTR are located in their genomes.

Figure 33.14
Restriction fragment length polymorphism of variable number tandem repeats (VNTR). For each person, a pair of homologous chromosomes is shown.

fewer fragments are produced by enzymic cleavage. An individual who is heterozygous for a polymorphism has a sequence variation in the DNA of one chromosome and not in the homologous chromosome. In such individuals, each chromosome can be traced from parent to offspring by determining the presence or absence of the polymorphism.

C. Prenatal diagnosis

Families with a history of severe genetic disease, such as an affected previous child or near relative, may wish to determine the presence of the disorder in a developing fetus. Prenatal diagnosis, in association with genetic counseling, allows for an informed reproductive decision if the fetus is affected.

1. **Methods available:** The available diagnostic methods vary in sensitivity and specificity. Visualization of the fetus, for example, by ultrasound or fiberoptic devices (fetoscopy), is useful only if the genetic abnormality results in gross anatomic defects (for example, neural tube defects). The chemical composition of the amniotic fluid can also provide diagnostic clues. For example, the presence of high levels of a-fetoprotein is associated with neural tube defects. Fetal cells obtained from amniotic fluid or from biopsy of the chorionic villi can be used for karyotyping, which assesses the morphology of metaphase chromosomes. Staining and cell sorting techniques permit the rapid identification of trisomies and translocations that produce an extra chromosome or chromosomes of abnormal lengths. However, molecular analysis of fetal DNA provides the most detailed genetic picture.

2. **Sources of DNA:** DNA may be obtained from white blood cells, amniotic fluid, or chorionic villi (Figure 33.15). For amniotic fluid, it used to be necessary to grow cells in culture for two to three weeks in order to have sufficient DNA for analysis. The ability to amplify DNA by PCR (see p. 479) has dramatically shortened the time needed for a DNA analysis.

3. **Direct diagnosis of sickle cell anemia using RFLP:** The genetic disorders of hemoglobin (Hb) are the most common genetic diseases in humans. In the case of sickle cell anemia (Figure 33.16),

the mutation that gives rise to the disease is actually one and the same mutation that gives rise to the polymorphism. Direct detection by RFLP of diseases that result from point mutations is, however, limited to only a few genetic diseases.

a. **Early efforts to diagnose sickle cell anemia:** In the past, prenatal diagnosis of hemoglobinopathies involved the determination of the amount and kinds of Hb synthesized in red cells obtained from fetal blood. However, the invasive procedures to obtain fetal blood have a high mortality rate (approximately 5%), and diagnosis cannot be carried out until late in the second trimester of pregnancy when HbS begins to be produced.

b. **RFLP analysis:** Sickle cell anemia is an example of a genetic disease caused by a point mutation (see p. 35). The sequence altered by the mutation abolishes the recognition site of the restriction endonuclease MstII: CCTNAGG (where N is any nucleotide; see Figure 33.16). Thus, the A-to-T mutation in codon 6 of the b^S-globin gene eliminates a cleavage site for the enzyme. Normal DNA digested with MstII yields a 1.15-kb fragment, whereas a 1.35-kb fragment is generated from the b^S gene as a result of the loss of one MstII cleavage site. Diagnostic techniques that allow analysis of fetal DNA from amniotic cells or chorionic villus sampling rather than fetal blood have proved valuable because they provide safe, early detection of sickle cell anemia as well as other genetic diseases. [Note: Genetic disorders caused by insertions or deletions between two restriction sites, rather than by the creation or loss of cleavage sites, will also display RFLP.]

4. **Indirect, prenatal diagnosis of phenylketonuria using RFLP:** The gene for phenylalanine hydroxylase (PAH), deficient in phenylketonuria ([PKU] see p. 270), is located on chromosome 12. It spans about 90 kb of genomic DNA and contains 13 exons separated by introns (Figure 33.17; see p. 426 for a description of exons and introns). Mutations in PAH usually do not directly affect any restriction endonuclease recognition site. To establish a diagnostic protocol for this disease, DNA of family members of the affected individual must be analyzed. The goal is to identify genetic markers (RFLPs) that are tightly linked to the disease trait. Once these markers are identified, RFLP analysis can be used to carry out prenatal diagnosis.

a. **Identification of the gene:** Determinining the presence of the mutant gene by identifying the polymorphism marker can be done if two conditions are satisfied. First, if the polymorphism is closely linked to a disease-producing mutation, the defective gene can be traced by detection of the RFLP. For example, if DNA from a family carrying a disease-causing gene is examined by restriction enzyme cleavage and Southern blotting, it is sometimes possible to find an RFLP that is consistently associated with that gene (that is, they show close linkage and are coinherited). It is then possible to trace the inheritance of the gene within a family without knowledge of the nature of the genetic defect or its precise location in the genome. [Note: The

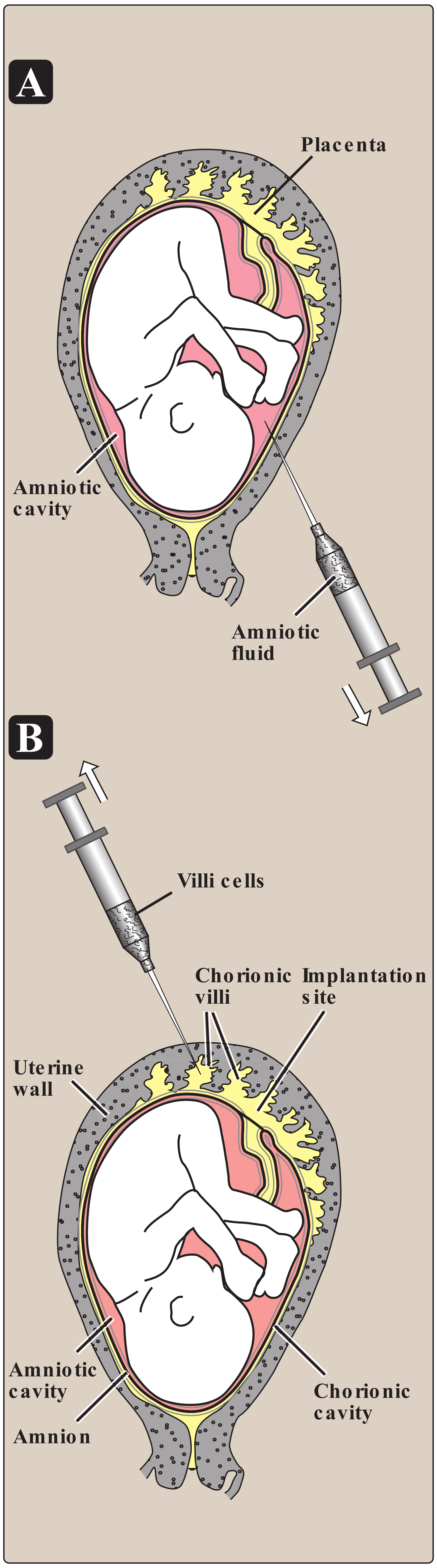
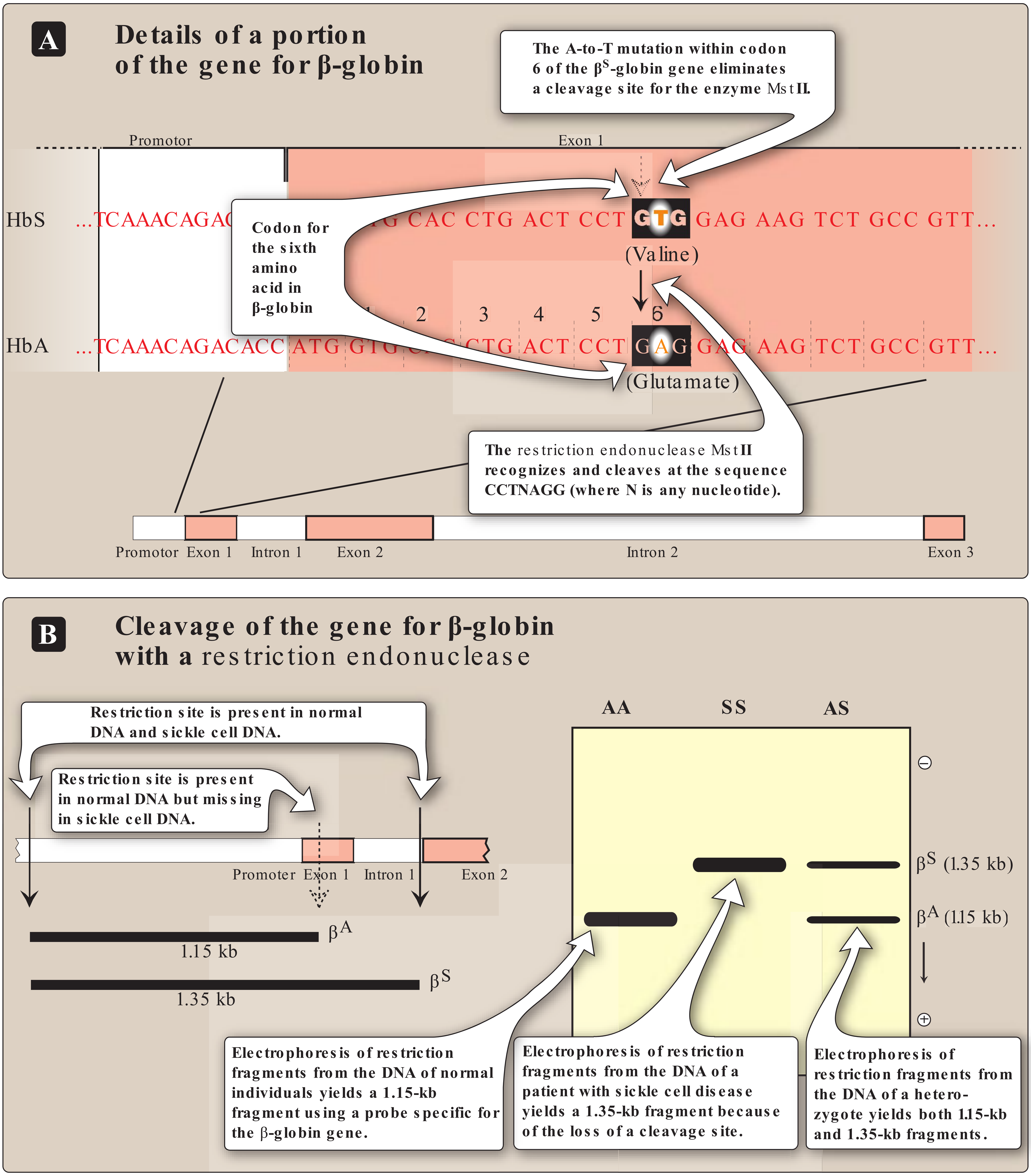


Figure 33.15
Sampling of fetal cells. A. Amniotic fluid. B. Chorionic villus.



A Details of a portion of the gene for β-globin

The A-to-T mutation within codon 6 of the β^S-globin gene eliminates a cleavage site for the enzyme Mst II.

Codon for the sixth amino acid in β-globin

The restriction endonuclease Mst II recognizes and cleaves at the sequence CCTNAGG (where N is any nucleotide).

Promotor
Exon 1
HbS
...TCAAACAGAC
G CAC CTG ACT CCT GTG GAG AAG TCT GCC GTT...
(Valine)
2
3
4
5
6
HbA
...TCAAACAGACACC ATG GTG C
CTG ACT CCT GAG GAG AAG TCT GCC GTT...
(Glutamate)
Promotor
Exon 1
Intron 1
Exon 2
Intron 2
Exon 3

B Cleavage of the gene for β-globin with a restriction endonuclease

Restriction site is present in normal DNA and sickle cell DNA.

Restriction site is present in normal DNA but missing in sickle cell DNA.

Electrophoresis of restriction fragments from the DNA of normal individuals yields a 1.15-kb fragment using a probe specific for the β-globin gene.

Electrophoresis of restriction fragments from the DNA of a patient with sickle cell disease yields a 1.35-kb fragment because of the loss of a cleavage site.

Electrophoresis of restriction fragments from the DNA of a heterozygote yields both 1.15-kb and 1.35-kb fragments.

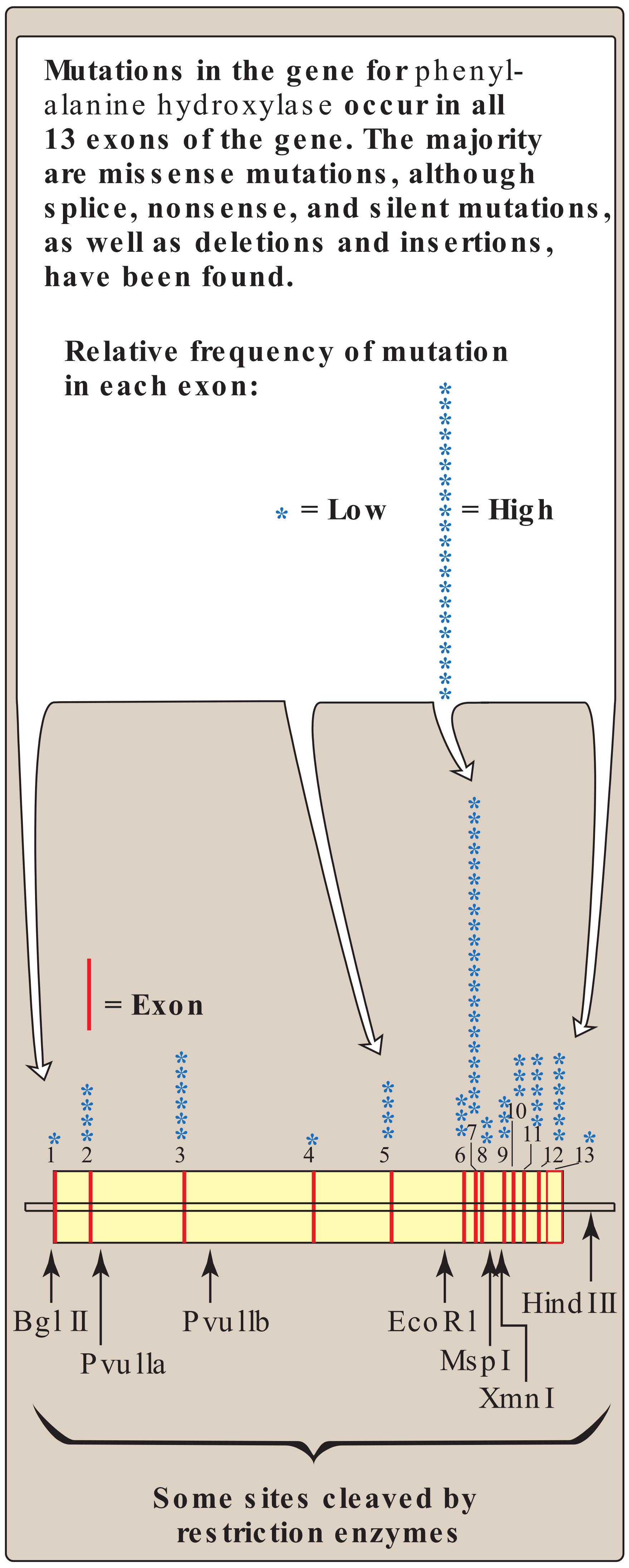
Promoter
Exon 1
Intron 1
Exon 2
β^A
1.15 kb
β^S
1.35 kb
AA
SS
AS
⊖
⊕
β^S (1.35 kb)
β^A (1.15 kb)

Figure 33.16
Detection of β^S-globin mutation. kb = kilobase (1 kb = 1000 base pairs in double-stranded DNA); Hb = hemoglobin.

polymorphism may be known from the study of other families with the disorder or may be discovered to be unique in the family under investigation.] Second, for autosomal recessive disorders, such as PKU, the presence of an affected individual in the family would aid in the diagnosis. This individual would have the mutation present on both chromosomes, allowing identification of the RFLP associated with the genetic disorder.

b. **RFLP analysis:** The presence of abnormal genes for PAH can be shown using DNA polymorphisms as markers to distinguish between normal and mutant genes. For example, Figure 33.18 shows a typical pattern obtained when DNA from the white blood cells of a family is cleaved with an appropriate restriction enzyme and subjected to electrophoresis. The vertical arrows represent the cleavage sites for the restriction enzyme used. The presence of a polymorphic site creates fragment “b” in the autoradiogram (after hybridization with a labeled PAH-cDNA probe), whereas the absence of this site yields only fragment “a.” Note that Subject II-2 demonstrates that the polymorphism, as shown by the presence of fragment “b,” is associated with the mutant gene. Therefore, in this particular family, the appearance of fragment “b” corresponds to the presence of a polymorphic site that marks the abnormal gene for PAH. The absence of fragment “b” corresponds to having only the normal gene. In Figure 33.18, examination of fetal DNA shows that the fetus inherited two abnormal genes from its parents and, therefore, has PKU.

c. **Value of DNA testing:** DNA-based testing is useful not only in determining if an unborn fetus is affected by PKU, but also in detecting unaffected carriers of the mutated gene to aid in family planning. [Note: PKU is treatable by dietary restriction of phenylalanine. Early diagnosis and treatment are essential in preventing severe neurologic damage in affected individuals.].



Mutations in the gene for phenylalanine hydroxylase **occur in all 13 exons of the gene. The majority are missense mutations, although splice, nonsense, and silent mutations, as well as deletions and insertions, have been found.**

Relative frequency of mutation in each exon:

Some sites cleaved by restriction enzymes

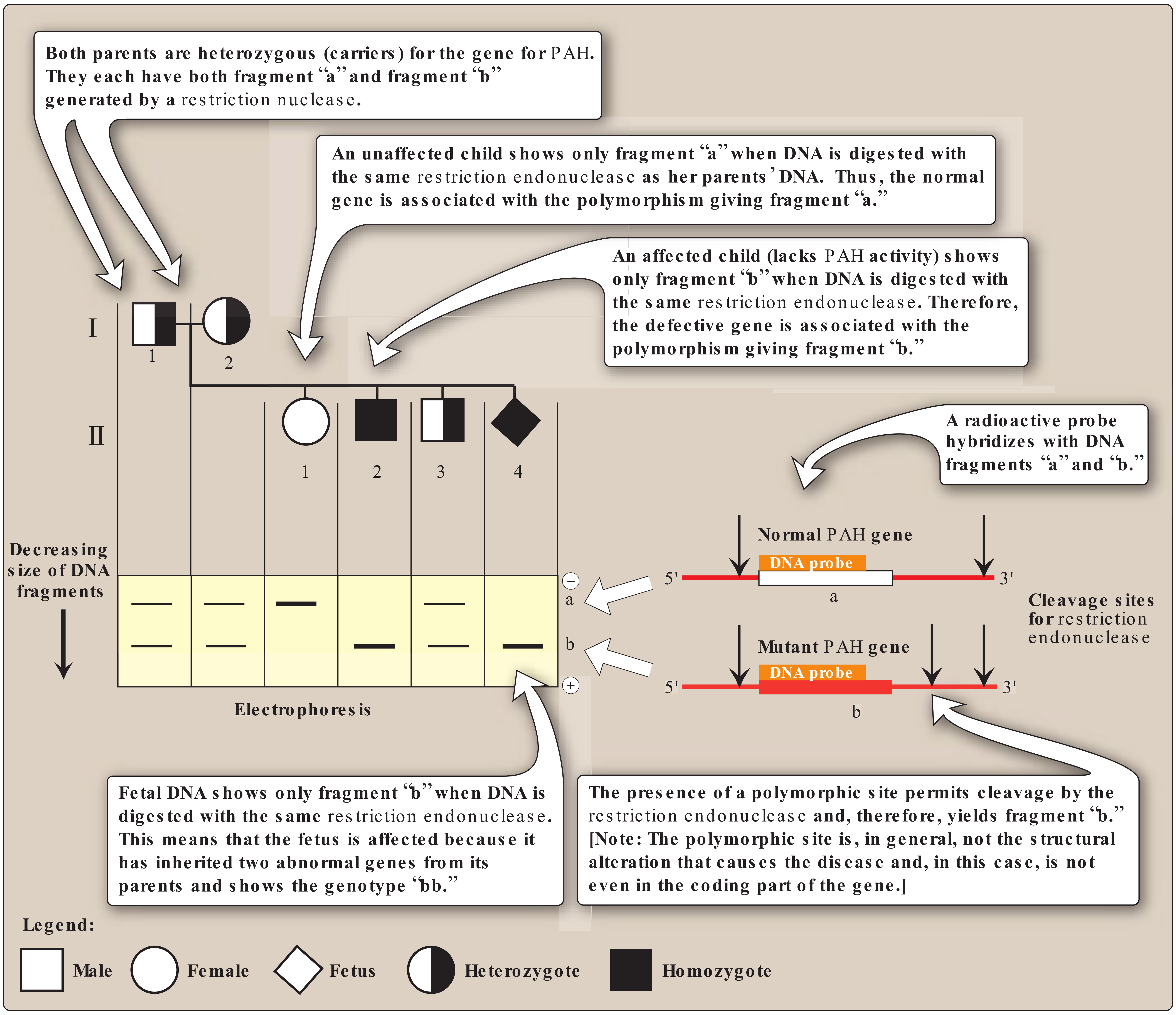
Figure 33.17
The gene for phenylalanine hydroxylase showing 13 exons, restriction sites, and some of the mutations resulting in phenylketonuria.

VII. POLYMERASE CHAIN REACTION

PCR is a test tube method for amplifying a selected DNA sequence that does not rely on the biologic cloning method described on p. 467. PCR permits the synthesis of millions of copies of a specific nucleotide sequence in a few hours. It can amplify the sequence, even when the targeted sequence makes up less than one part in a million of the total initial sample. The method can be used to amplify DNA sequences from any source, including viral, bacterial, plant, or animal. The steps in PCR are summarized in Figures 33.19 and 33.20.

A. Steps of a polymerase chain reaction

PCR uses DNA polymerase to repetitively amplify targeted portions of genomic or cDNA. Each cycle of amplification doubles the amount of DNA in the sample, leading to an exponential increase (2^n, where n = cycle number) in DNA with repeated cycles of amplification. The amplified DNA products can then be separated by gel electrophoresis, detected by Southern blotting and hybridization, and sequenced.



Both parents are heterozygous (carriers) for the gene for PAH. They each have both fragment "a" and fragment "b" generated by a restriction nuclease.

An unaffected child shows only fragment "a" when DNA is digested with the same restriction endonuclease as her parents' DNA. Thus, the normal gene is associated with the polymorphism giving fragment "a."

An affected child (lacks PAH activity) shows only fragment "b" when DNA is digested with the same restriction endonuclease. Therefore, the defective gene is associated with the polymorphism giving fragment "b."

A radioactive probe hybridizes with DNA fragments "a" and "b."

Fetal DNA shows only fragment "b" when DNA is digested with the same restriction endonuclease. This means that the fetus is affected because it has inherited two abnormal genes from its parents and shows the genotype "bb."

The presence of a polymorphic site permits cleavage by the restriction endonuclease and, therefore, yields fragment "b." [Note: The polymorphic site is, in general, not the structural alteration that causes the disease and, in this case, is not even in the coding part of the gene.]

Figure 33.18
Analysis of restriction fragment length polymorphism in a family with a child affected by phenylketonuria (PKU), an autosomal recessive disease. The molecular defect in the gene for phenylalanine hydroxylase (PAH) in the family is not known. The family wanted to know if the current pregnancy would be affected by PKU.

1. **Primer construction:** It is not necessary to know the nucleotide sequence of the target DNA in the PCR method. However, it is necessary to know the nucleotide sequence of short segments on each side of the target DNA. These stretches, called flanking sequences, bracket the DNA sequence of interest. The nucleotide sequences of the flanking regions are used to construct two, single-stranded oligonucleotides, usually 20–35 nucleotides long, which are complementary to the respective flanking sequences. The 3'-hydroxyl end of each oligonucleotide points toward the target sequence (see Figure 33.19). These synthetic oligonucleotides function as primers in PCR reactions.

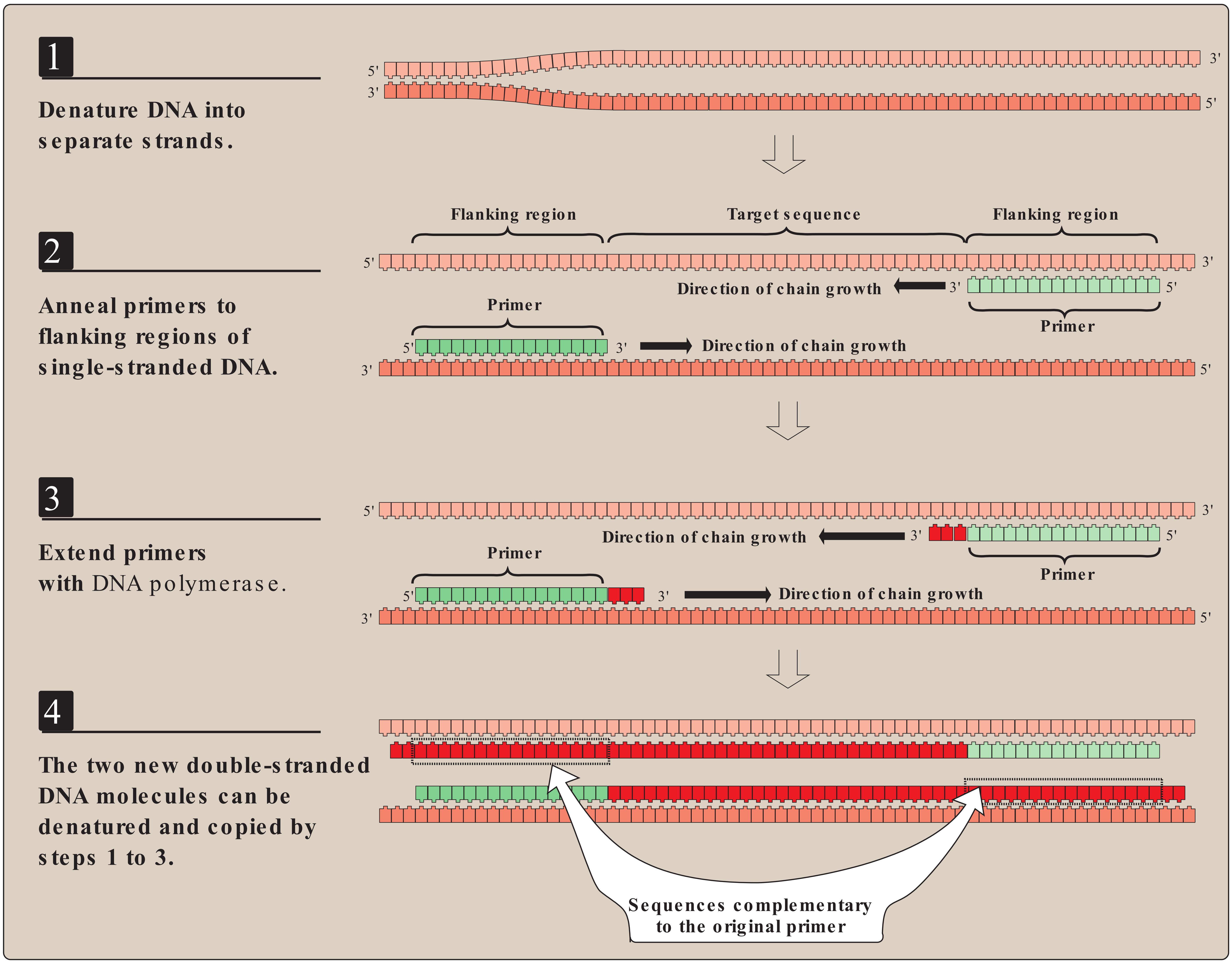

1
Denature DNA into separate strands.
5'
3'
3'
5'
2
Anneal primers to flanking regions of single-stranded DNA.
Flanking region
Target sequence
Flanking region
5'
3'
Direction of chain growth
3'
5'
Primer
Primer
5'
3'
Direction of chain growth
3'
5'
3
Extend primers with DNA polymerase.
5'
3'
Direction of chain growth
3'
5'
Primer
Primer
5'
3'
Direction of chain growth
3'
5'
4
The two new double-stranded DNA molecules can be denatured and copied by steps 1 to 3.
Sequences complementary to the original primer

Figure 33.19
Steps in one cycle of the polymerase chain reaction.

2. **Denature the DNA:** The DNA to be amplified is heated to separate the double-stranded target DNA into single strands.

3. **Annealing of primers to single-stranded DNA:** The separated strands are cooled and allowed to anneal to the two primers (one for each strand).

4. **Chain extension:** DNA polymerase and deoxyribonucleoside triphosphates (in excess) are added to the mixture to initiate the synthesis of two new strands complementary to the original DNA strands. DNA polymerase adds nucleotides to the 3'-hydroxyl end of the primer, and strand growth extends across the target DNA, making complementary copies of the target. [Note: PCR products can be several thousand bp long.] At the completion of one cycle of replication, the reaction mixture is heated again to

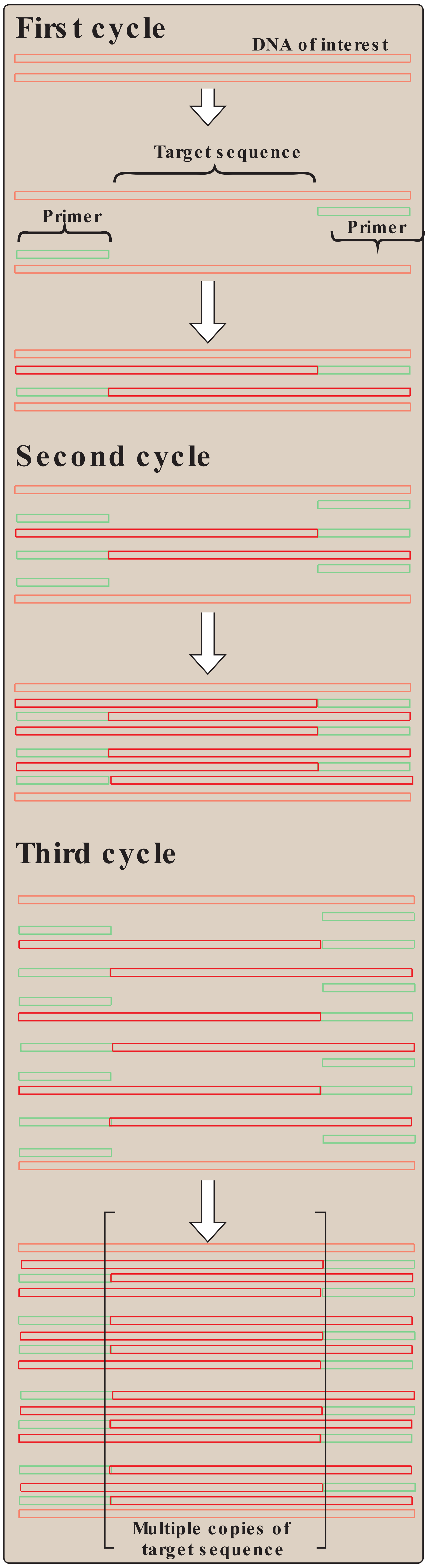


Figure 33.20
Multiple cycles of polymerase chain reaction.

separate the strands (of which there are now four). Each strand binds a complementary primer, and the cycle of chain extension is repeated. By using a heat-stable DNA polymerase (for example, Taq polymerase from the bacterium, Thermus aquaticus that normally lives at high temperatures), the polymerase is not denatured and, therefore, does not have to be added at each successive cycle. Typically 20–30 cycles are run during this process, amplifying the DNA by a million-fold (2^{20}) to a billion-fold (2^{30}). [Note: Each extension product includes a sequence at its 5'-end that is complementary to the primer (see Figure 33.19). Thus, each newly synthesized strand can act as a template for the successive cycles (see Figure 33.20). This leads to an exponential increase in the amount of target DNA with each cycle, hence, the name "polymerase chain reaction."] Probes can be made during PCR by adding labeled nucleotides to the last few cycles.

B. Advantages of polymerase chain reaction

The major advantages of PCR over biologic cloning as a mechanism for amplifying a specific DNA sequence are sensitivity and speed. DNA sequences present in only trace amounts can be amplified to become the predominant sequence. PCR is so sensitive that DNA sequences present in an individual cell can be amplified and studied. Isolating and amplifying a specific DNA sequence by PCR is faster and less technically difficult than traditional cloning methods using recombinant DNA techniques.

C. Applications

PCR has become a very common tool for a large number of applications.

1. **Comparison of a normal gene with a mutant form of the gene:** PCR allows the synthesis of mutant DNA in sufficient quantities for a sequencing protocol without laboriously cloning the altered DNA.

2. **Detection of low-abundance nucleic acid sequences:** Viruses that have a long latency period, such as human immunodeficiency virus (HIV), are difficult to detect at the early stage of infection using conventional methods. PCR offers a rapid and sensitive method for detecting viral DNA sequences even when only a small proportion of cells harbors the virus. [Note: Quantitative real time PCR (qRT-PCR) allows quantification of starting amounts of the target nucleic acid as PCR progresses (in real time) rather than at the end and is useful in determining viral load (the amount of virus).]

3. **Forensic analysis of DNA samples:** DNA fingerprinting by means of PCR has revolutionized the analysis of evidence from crime scenes. DNA isolated from a single human hair, a tiny spot of blood, or a sample of semen is sufficient to determine whether the sample comes from a specific individual. The DNA markers analyzed for such fingerprinting are most commonly short tandem repeat polymorphisms. These are very similar to the VNTRs described previously (see p. 475) but are smaller in size. [Note: Determination of paternity uses the same techniques.]

4. **Prenatal diagnosis and carrier detection of cystic fibrosis:** Cystic fibrosis is an autosomal recessive genetic disease resulting from mutations in the gene for the cystic fibrosis transmembrane conductance regulator (CFTR) protein. The most common mutation is a three-base deletion that results in the loss of a phenylalanine residue from the CFTR protein (see p. 434). Because the mutant allele is three bases shorter than the normal allele, it is possible to distinguish them from each other by the size of the PCR products obtained by amplifying that portion of the DNA. Figure 33.21 illustrates how the results of such a PCR test can distinguish between homozygous normal, heterozygous (carriers), and homozygous mutant (affected) individuals.

> The simultaneous amplification of multiple regions of a target DNA using multiple primer pairs is known as multiplex PCR. It allows detection of the loss of 1 or more exons in a gene with many exons such as the gene for CFTR, which has 27 exons.

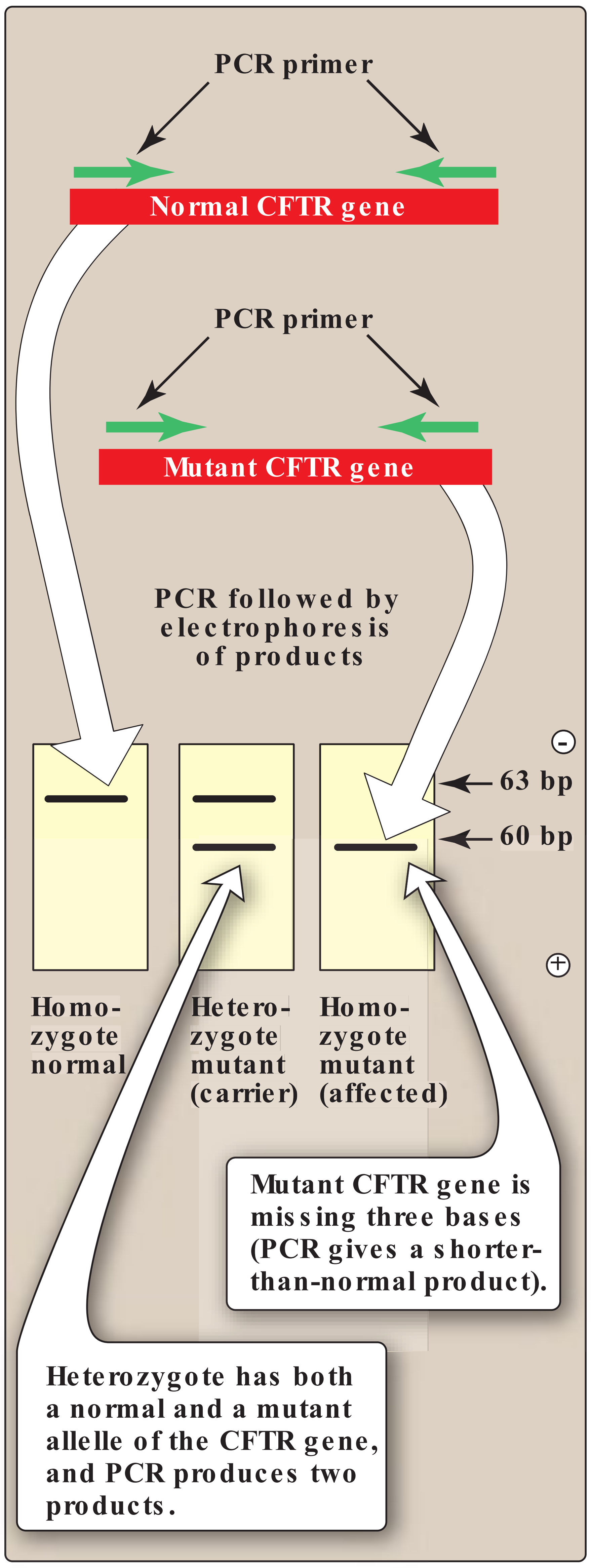

PCR primer
Normal CFTR gene
PCR primer
Mutant CFTR gene
PCR followed by electrophoresis of products
−
+
63 bp
60 bp
Homozygote normal
Heterozygote mutant (carrier)
Homozygote mutant (affected)
Mutant CFTR gene is missing three bases (PCR gives a shorter-than-normal product).
Heterozygote has both a normal and a mutant allele of the CFTR gene, and PCR produces two products.

Figure 33.21
Genetic testing for cystic fibrosis using the polymerase chain reaction (PCR). CFTR = cystic fibrosis transmembrane conductance regulator; bp = base pairs.

VIII. ANALYSIS OF GENE EXPRESSION

The tools of biotechnology not only allow the study of gene structure, but also provide ways of analyzing the mRNA and protein products of gene expression.

A. Determination of messenger RNA levels

mRNA levels are usually determined by the hybridization of labeled probes to either mRNA itself or to cDNA produced from mRNA. [Note: Amplification of cDNA made from mRNA by retroviral reverse transcriptase (RT) is referred to as RT-PCR.]

1. **Northern blots:** Northern blots are very similar to Southern blots (see Figure 33.12, p. 474), except that the original sample contains a mixture of mRNA molecules that are separated by electrophoresis, then transferred to a membrane and hybridized to a radiolabeled probe. The bands obtained by autoradiography give a measure of the amount and size of particular mRNA molecules in the sample.

2. **Microarrays:** DNA microarrays contain thousands of immobilized ssDNA sequences organized in an area no larger than a microscope slide. These microarrays are used to analyze a sample for the presence of gene variations or mutations (genotyping) or to determine the patterns of mRNA production (gene expression analysis), analyzing thousands of genes at the same time. For genotyping analysis, the sample is from genomic DNA. For expression analysis, the population of mRNA molecules from a particular cell type is converted to cDNA and labeled with a fluorescent tag (Figure 33.22). This mixture is then exposed to a gene (DNA) chip, which is a glass slide or membrane containing thousands of tiny spots of DNA, each corresponding to a different gene. The amount

of fluorescence bound to each spot is a measure of the amount of that particular mRNA in the sample. DNA microarrays are used to determine the differing patterns of gene expression in two different types of cell (for example, normal and cancer cells; see Figure 33.22). They can also be used to subclassify cancers, such as breast cancer, to optimize treatment. [Note: Microarrays involving proteins and the antibodies or other proteins that recognize them are being used to identify biomarkers to aid in the diagnosis, prognosis, and treatment of disease based on a patient's protein expression profile. Protein (and DNA) microarrays are important tools in the development of personalized medicine.]

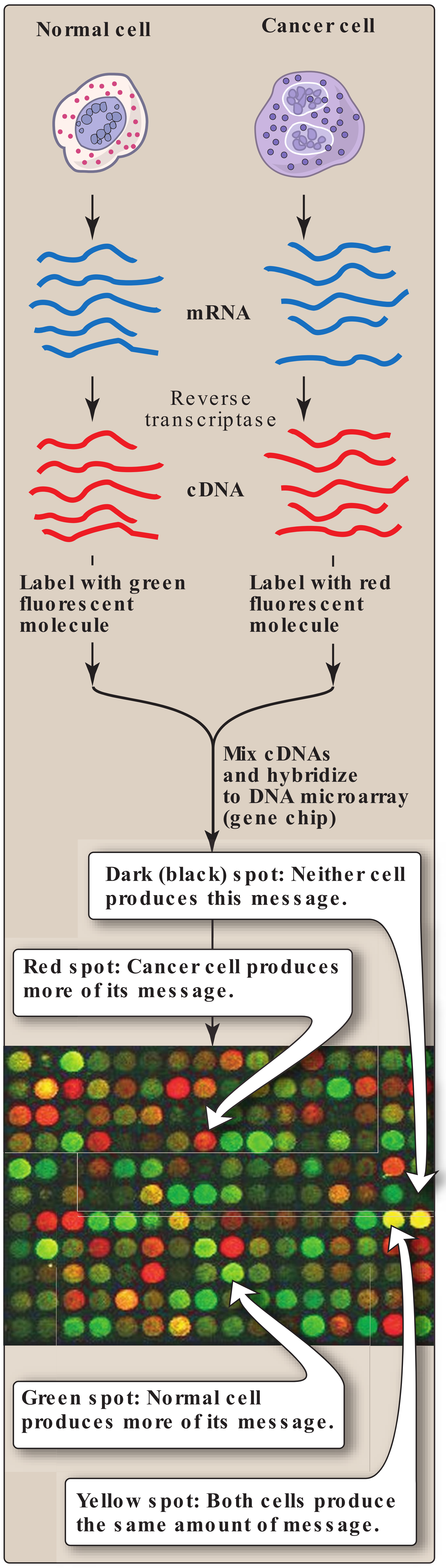

Normal cell
Cancer cell
mRNA
Reverse transcriptase
cDNA
Label with green fluorescent molecule
Label with red fluorescent molecule
Mix cDNAs and hybridize to DNA microarray (gene chip)
Dark (black) spot: Neither cell produces this message.
Red spot: Cancer cell produces more of its message.
Green spot: Normal cell produces more of its message.
Yellow spot: Both cells produce the same amount of message.

Figure 33.22
Microarray analysis of gene expression using DNA (gene) chips. [Note: Protein chips are also used.] mRNA = messenger RNA; cDNA = complementary DNA.

B. Analysis of proteins

The kinds and amounts of proteins in cells do not always directly correspond to the amounts of mRNA present. Some mRNAs are translated more efficiently than others, and some proteins undergo posttranslational modification. When analyzing the abundance and interactions of a large number of proteins, automated methods involving a variety of techniques, such as mass spectrometry and two-dimensional electrophoresis, are used. When investigating one, or a limited number of proteins, labeled antibodies are used to detect and quantify specific proteins and to determine posttranslational modifications.

1. **Enzyme-linked immunosorbent assays (ELISAs):** These assays are performed in the wells of a plastic microtiter dish. The antigen (protein) is bound to the plastic of the dish. The probe used consists of an antibody specific for the particular protein to be measured. The antibody is covalently bound to an enzyme, which will produce a colored product when exposed to its substrate. The amount of color produced is proportional to the amount of antibody present and, indirectly, to the amount of protein in a test sample.

2. **Western blots:** Western blots (also called immunoblots) are similar to Southern blots, except that protein molecules in the sample are separated by electrophoresis and blotted (transferred) to a membrane. The probe is a labeled antibody, which produces a band at the location of its antigen.

3. **Detecting exposure to human immunodeficiency virus (HIV):** ELISA and Western blots are commonly used to detect exposure to HIV by measuring the amount of anti-HIV antibodies present in a patient's blood sample. ELISAs are used as the primary screening tool, because they are very sensitive. Because these assays sometimes give false positives, however, Western blots, which are more specific, are often used as a confirmatory test (Figure 33.23). [Note: ELISA and Western blots can only detect HIV exposure after anti-HIV antibodies appear in the bloodstream. PCR-based testing for HIV is more useful in the first few months after exposure.]

C. Proteomics

The study of the proteome or all the proteins expressed by a genome, including their relative abundance, distribution, posttranslational modifications, functions, and interactions with other macromolecules, is known as proteomics. The 20,000 to 25,000 protein-coding genes of the human genome translate into well over 100,000 proteins when posttranscriptional and posttranslational modifications are considered. Although a genome remains essentially unchanged, the amounts and types of proteins in any particular cell change dramatically as genes are turned on and off. [Note: Proteomics (and genomics) required the parallel development of bioinformatics, the computer-based organization, storage, and analysis of biologic data.] Figure 33.24 compares some of the analytic techniques discussed in this chapter.

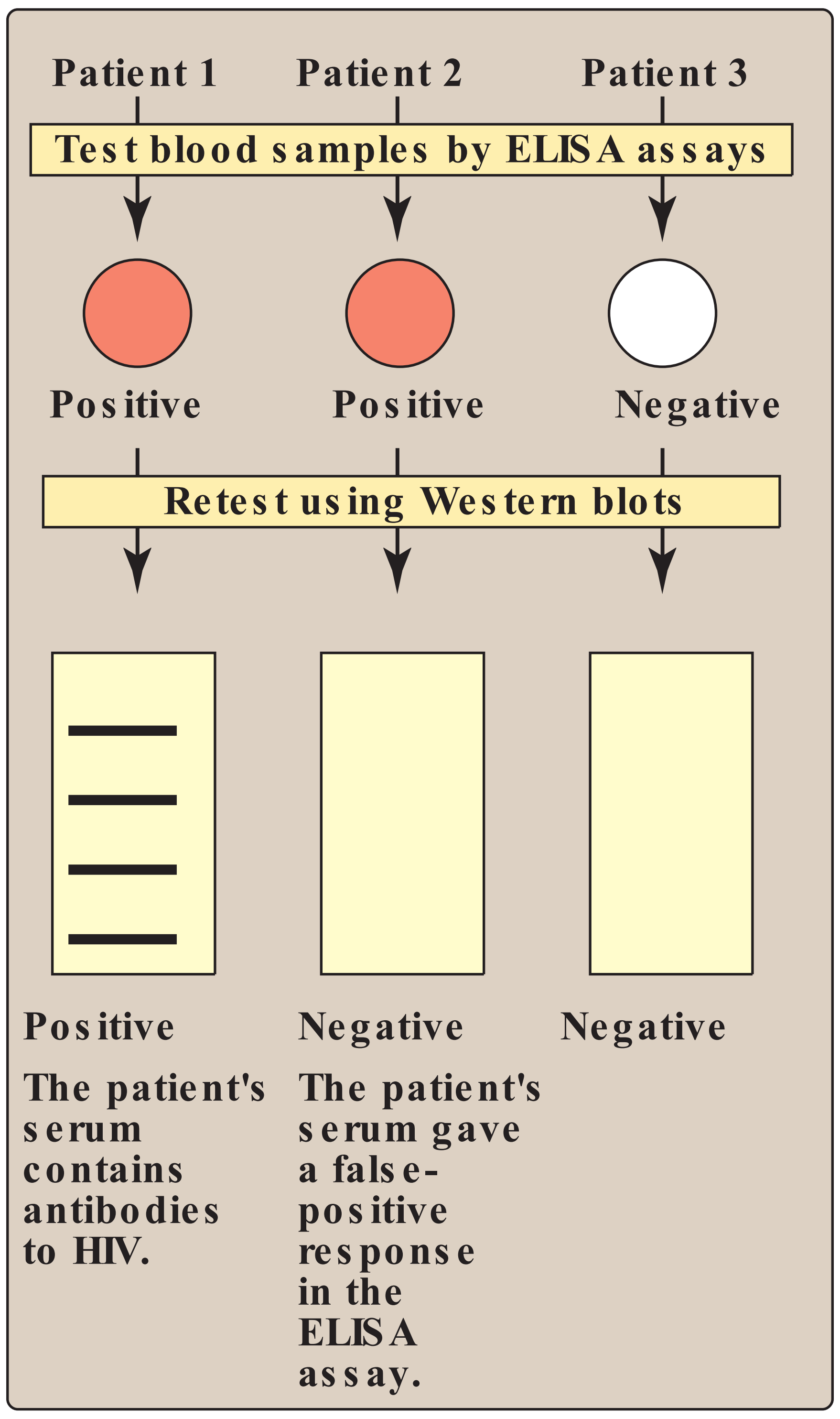

Patient 1
Patient 2
Patient 3
Test blood samples by ELISA assays
Positive
Positive
Negative
Retest using Western blots
Positive
Negative
Negative
The patient's serum contains antibodies to HIV.
The patient's serum gave a false-positive response in the ELISA assay.

Figure 33.23
Testing for HIV exposure by enzyme-linked immunosorbent assays (ELISAs) and Western blots.

IX. GENE THERAPY

The goal of gene therapy is to treat disease through insertion of the normal, cloned DNA for a gene into the somatic cells of a patient who has a defect in that gene as a result of a disease-causing mutation. Because somatic gene therapy changes only the targeted somatic cells, the change is not passed on to the next generation. [Note: In germline gene therapy, it is the germ cells that are modified, and so the change is passed on. A long-standing moratorium on germline gene therapy is in effect world-wide.] There are two types of gene transfer: 1) ex vivo, in which cells from the patient are removed, transduced, and returned; and 2) in vivo, in which the cells are directly transduced. Both types require use of a vector (viral or nonviral) to deliver the DNA into the target cell. Challenges of gene therapy include development of vectors, achievement of long-lived expression, and prevention of side effects such as an immune response. The first successful gene therapy (1990) involved two patients with severe combined immunodeficiency disease (SCID) caused by mutations to the gene for adenosine deaminase (see p. 301). It utilized mature T lymphocytes transduced ex vivo with a viral vector (Figure 33.25). Since 1990, only a small number of patients (with a variety of disorders, such as hemophilia, cancers, and certain types of blindness) have been treated with gene therapy, with varying degrees of success.

TECHNIQUE	SAMPLE ANALYZED	GEL USED	PURPOSE
Southern blot	DNA	Yes	Detects large changes in DNA; detects point mutations that create or destroy restriction sites
Northern blot	RNA	Yes	Measures mRNA amounts and sizes
Western blot	Protein	Yes	Measures protein amounts
ASO	DNA	No	Detects small changes in DNA
Microarray	cDNA or genomic DNA	No	Measures many mRNA levels at once; detects genomic changes
	Protein	No	Measures protein expression levels and protein–protein interactions
ELISA	Proteins	No	Detects proteins (antigens) or antibodies

Figure 33.24
Techniques used to analyze DNA, RNA, and proteins. ASO = allele-specific oligonucleotides. ELISA = enzyme-linked immunosorbent assay; cDNA = complementary DNA; mRNA = messenger RNA.

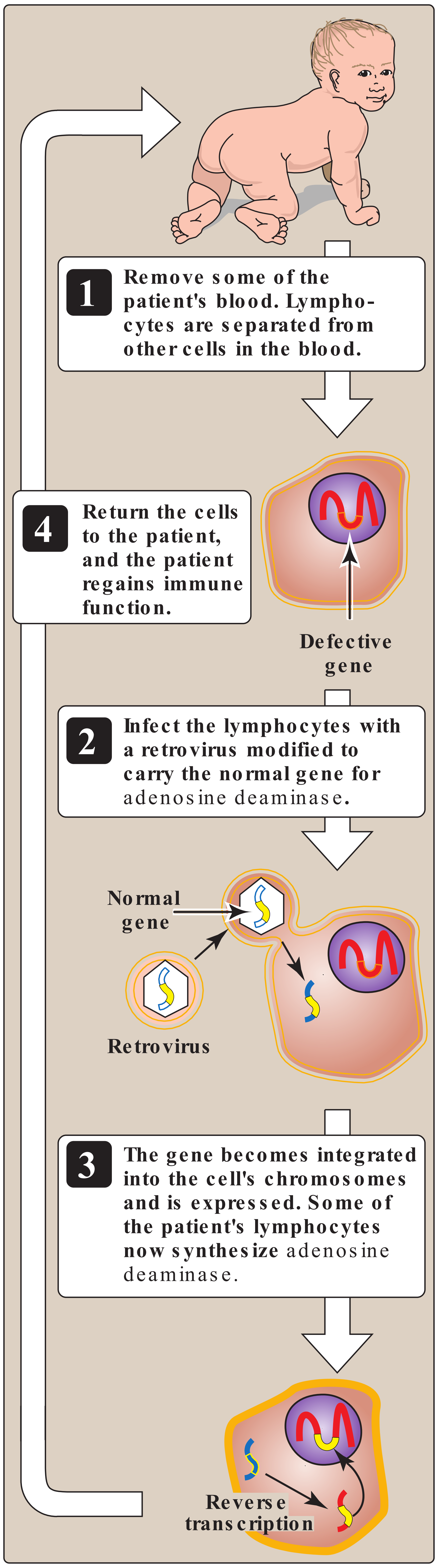

1
Remove some of the patient's blood. Lymphocytes are separated from other cells in the blood.
4
Return the cells to the patient, and the patient regains immune function.
Defective gene
2
Infect the lymphocytes with a retrovirus modified to carry the normal gene for adenosine deaminase.
Normal gene
Retrovirus
3
The gene becomes integrated into the cell's chromosomes and is expressed. Some of the patient's lymphocytes now synthesize adenosine deaminase.
Reverse transcription

Figure 33.25
Gene therapy for SCID caused by adenosine deaminase deficiency. [Note: Bone marrow stem cells and a modified retroviral vector are now used.]

X. TRANSGENIC ANIMALS

Transgenic animals can be produced by injecting a cloned gene into the fertilized egg. If the gene becomes successfully integrated into a chromosome, it will be present in the germline of the resulting animal and can be passed along from generation to generation. A giant mouse called "Supermouse" was produced in this way by injecting the gene for rat growth hormone into a fertilized mouse egg. [Note: Transgenic animals have been designed that produce human proteins in their milk. Antithrombin, an anticlotting protein, was produced by transgenic goats and approved for clinical use in 2009 (see online Chapter 34).] Sometimes, rather than introducing a functional gene into a mouse, a nonfunctional version is inserted. Such genetically engineered animals can be used to produce a colony of "knockout mice" that lack the product of the affected gene. Such animals can then serve as models for the study of a corresponding human disease. [Note: Knock-in mice result if the inserted gene expresses a mutated product or under- or overexpresses a product.]

XI. CHAPTER SUMMARY

Restriction endonucleases are bacterial enzymes that cleave double-stranded DNA (dsDNA) into smaller fragments. Each enzyme cleaves at a specific four to eight–base pair sequence (a restriction site), producing DNA segments called **restriction fragments**. The sequences that are recognized are **palindromic**. Restriction enzymes form either **staggered cuts** (**sticky ends**) or **blunt-end cuts** on the DNA. **Bacterial** DNA ligases can join two DNA fragments from different sources if they have been cut by the same restriction endonuclease. This hybrid combination of two fragments is called a **recombinant DNA molecule**. Introduction of a foreign DNA molecule into a replicating cell permits the **amplification** (production of many copies) of the DNA, a process called **cloning**. A **vector** is a molecule of DNA to which the fragment of DNA to be cloned is joined. Vectors must be capable of **autonomous replication** within the host cell, must contain at least one specific nucleotide sequence recognized by a restriction endonuclease, and must carry at least one gene that confers the ability to select for the vector such as an **antibiotic resistance gene**. Prokaryotic organisms normally contain small, circular, extrachromosomal DNA molecules called **plasmids** that can serve as **vectors**. They can be readily isolated from the bacterium (or artificially constructed); joined with the DNA of interest; and reintroduced into the bacterium, which will replicate, thus making multiple copies of the **hybrid plasmid**. A **DNA library** is a collection of cloned restriction fragments of the DNA of an organism. **A genomic library** is a collection of fragments of dsDNA obtained by digestion of the total DNA of the organism with a restriction endonuclease and subsequent ligation to an appropriate vector. It ideally contains a copy of every DNA nucleotide sequence in the genome. In contrast, **complementary DNA (cDNA) libraries** contain only those DNA sequences that are complementary to messenger RNA (mRNA) molecules present in a cell and differ from one cell type to another. Because cDNA has no intervening sequences, it can be cloned into an **expression vector** for the synthesis of human proteins by bacteria or eukaryotes. Cloned, then purified, fragments of DNA can be sequenced, for example, using the **Sanger dideoxy method**. A **probe** is a small piece of RNA or single-stranded DNA (usually labeled with a radioisotope, such as ^{32}P, or another recognizable compound, such as biotin or a fluorescent dye) that has a nucleotide sequence complementary to the DNA molecule of interest (**target DNA**). Probes can be used to identify which clone of a library or which band on a gel contains the target DNA. **Southern blotting** is a technique that can be used to detect specific sequences present in DNA. The DNA is cleaved using a restriction endonuclease, and the pieces are separated by **gel electrophoresis** and are denatured and transferred (blotted) to a **nitrocellulose membrane** for analysis. The fragment of interest is detected using a **probe**. The human genome contains many thousands of polymorphisms (DNA sequence variations at a given locus). Polymorphisms can arise from single-base changes and from tandem repeats. A polymorphism can serve as a genetic marker that can be followed through families. A **restriction fragment length polymorphism** (**RFLP**) is a genetic variant that can be observed by cleaving the DNA into restriction fragments using a restriction enzyme. A base substitution in one or more nucleotides at a restriction site can render the site unrecognizable by a particular restriction endonuclease. A new restriction site also can be created by the same mechanism. In either case, cleavage with the endonuclease results in fragments of lengths differing from the normal that can be detected by DNA hybridization. This technique can be used to diagnose genetic diseases early in the gestation of a fetus. The **polymerase chain reaction** (**PCR**), another method for **amplifying** a selected DNA sequence, does not rely on the biologic cloning method. PCR permits the synthesis of millions of copies of a specific nucleotide sequence in a few hours. It can amplify the sequence, even when the targeted sequence makes up less than one part in a million of the total initial sample. The method can be used to amplify DNA sequences from any source. **Applications of the PCR technique** include: 1) efficient comparison of a normal gene with a mutant form of the gene, 2) detection of low-abundance nucleic acid sequences, 3) forensic analysis of DNA samples, and 4) prenatal diagnosis and carrier detection (for example, of cystic fibrosis). The **products of gene expression** (mRNA and proteins) can be measured by techniques such as the following: **Northern blots** are very similar to Southern blots except that the original sample contains a mixture of **mRNA** molecules that are separated by electrophoresis, then hybridized to a radiolabeled probe; **microarrays** are used to determine the differing patterns of gene expression in two different types of cells (for example, normal and cancer cells); **enzyme-linked immunosorbent assays** and **Western blots** (**immunoblots**) are used to detect specific proteins. **Proteomics** is the study of all the proteins expressed by a genome. The goal of **gene therapy** is the insertion of a normal cloned gene to replace a defective gene in a **somatic cell**. Insertion of a foreign gene into the germline of an animal creates a **transgenic animal** that can produce therapeutic proteins or serve as a model for human diseases.

Blood Clotting

34

I. OVERVIEW

Blood clotting (coagulation) is designed to rapidly stop bleeding from a damaged blood vessel in order to maintain a constant blood volume (hemostasis). Coagulation is accomplished through vasoconstriction and the formation of a clot (thrombus) that consists of a plug of platelets and a meshwork of the protein fibrin that stabilizes the platelet plug. Clotting occurs in association with membranes on the surface of platelets and damaged blood vessels (Figure 34.1). [Note: If clotting occurs within an intact vessel such that the lumen is occluded and blood flow is impeded, a condition known as thrombosis, serious tissue damage and even death can occur. This is what happens, for example, during a myocardial infarction (MI).] Processes to limit clot formation to the area of damage and remove the clot once vessel repair is underway also play essential roles in hemostasis. [Note: Separate discussions of the formation of the platelet plug and the fibrin meshwork facilitate presentation of these multistep, multicomponent processes. However, the two work together to maintain hemostasis.]

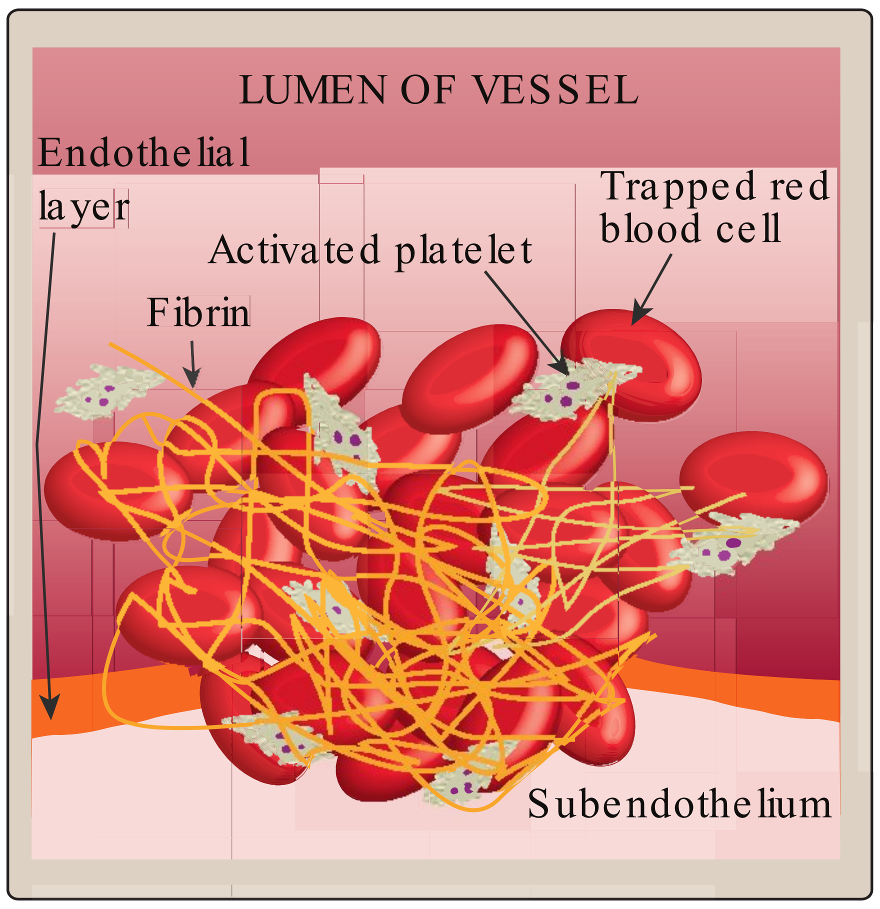


Figure 34.1
A blood clot formed by a plug of activated platelets and a meshwork of fibrin at the site of vessel injury.

II. FIBRIN MESHWORK FORMATION

The formation of the fibrin meshwork involves two unique pathways that converge to form a common pathway (Figure 34.2). In each pathway, the major components are proteins (called factors) designated by Roman numerals. The factors are glycoproteins that are synthesized and secreted by the liver, primarily. [Note: Several factors are also denoted by alternative names. For example, active factor X (FXa), the point of pathway convergence, is also known as Stuart factor.]

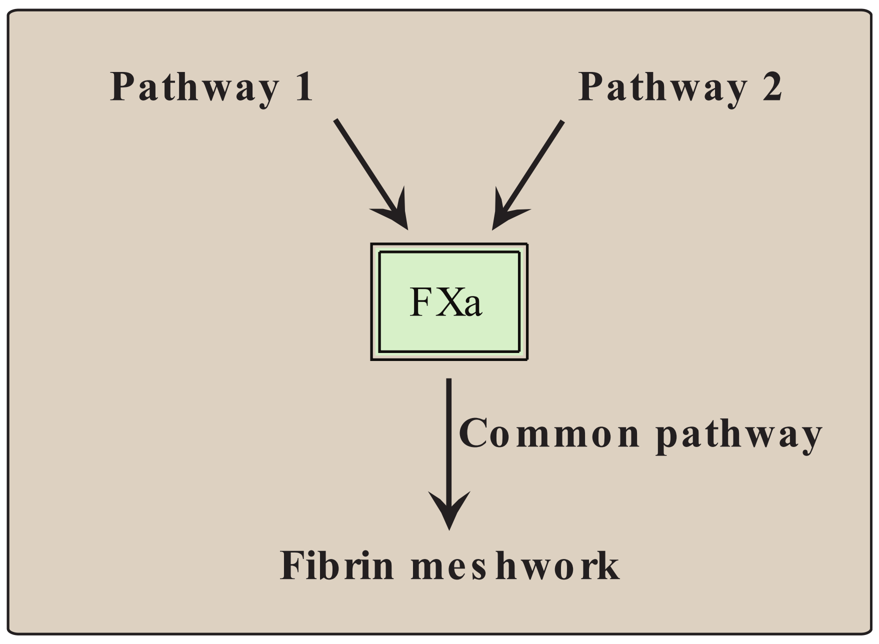


Figure 34.2
Three pathways involved in formation of the fibrin meshwork.

A. Protolytic cascade

Within the pathways, a cascade is set up in which proteins are converted from an inactive form, or zymogen, to an active form by proteolytic cleavage in which the protein product of one activation reaction initiates another. The active form of a factor is denoted by a lower case "a" after the numeral. The active proteins FIIa, FVIIa, FIXa, FXa, FXIa, and FXIIa are enzymes that function as serine proteases with trypsin-like specificity and, therefore, cleave

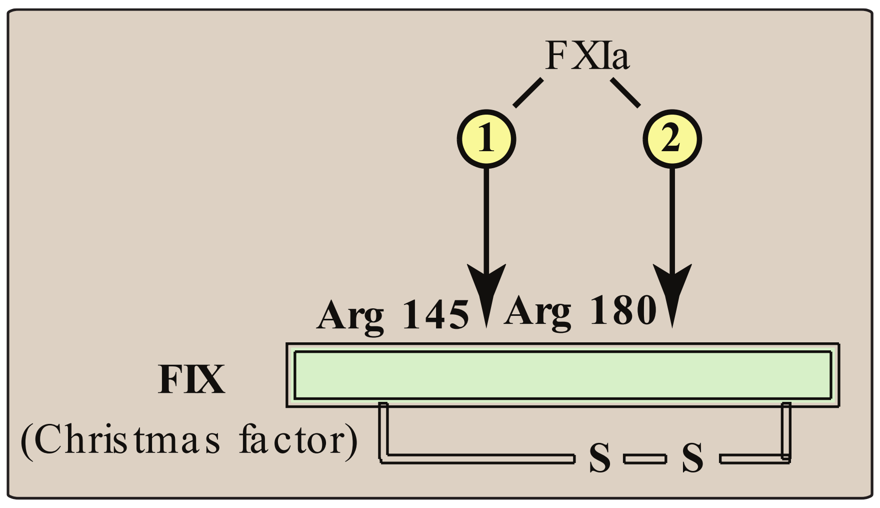


Figure 34.3
Activation of FIX (Christmas factor) via proteolysis by the serine protease FXIa. [Note: Activation can occur by conformational change for some of the factors.] a = active; Arg = arginine.

a peptide bond on the carboxyl side of an arginine or lysine residue in a polypeptide. For example, FIX (Christmas factor) is activated through cleavage at arginine 145 and arginine 180 by FXIa (Figure 34.3). The proteolytic cascade results in enormous rate acceleration, because one active protease can produce many molecules of active product each of which, in turn, can activate many molecules of the next protein in the cascade. In some cases, activation can be caused by a conformational change in the protein in the absence of proteolysis. [Note: Nonproteolytic proteins play a role as accessory proteins (cofactors) in the pathways. FIII, FV, and FVIII are the accessory proteins.]

B. Role of phosphatidylserine and calcium

The presence of the negatively charged phospholipid phosphatidylserine (PS) and positively charged calcium ions (Ca^{2+}) accelerates the rate of some steps in the cascade.

1. **Negatively charged phosphatidylserine:** PS is located primarily on the intracellular (cytosolic) face of the plasma membrane. Its exposure signals injury to the endothelial cells that line blood vessels. PS is also exposed on the surface of activated platelets.

2. **Calcium ions:** Ca^{2+} binds the negatively charged g-carboxyglutamate (Gla) residues present in certain clotting serine proteases (FII, FVII, FIX, and FX), facilitating the binding of these proteins to exposed phospholipids (Figure 34.4). The Gla residues are good chelators of Ca^{2+} because of their two adjacent negatively charged carboxylate groups (Figure 34.5). [Note: The use of chelating agents such as sodium citrate to bind Ca^{2+} in blood-collecting tubes or bags prevents the blood from clotting.]

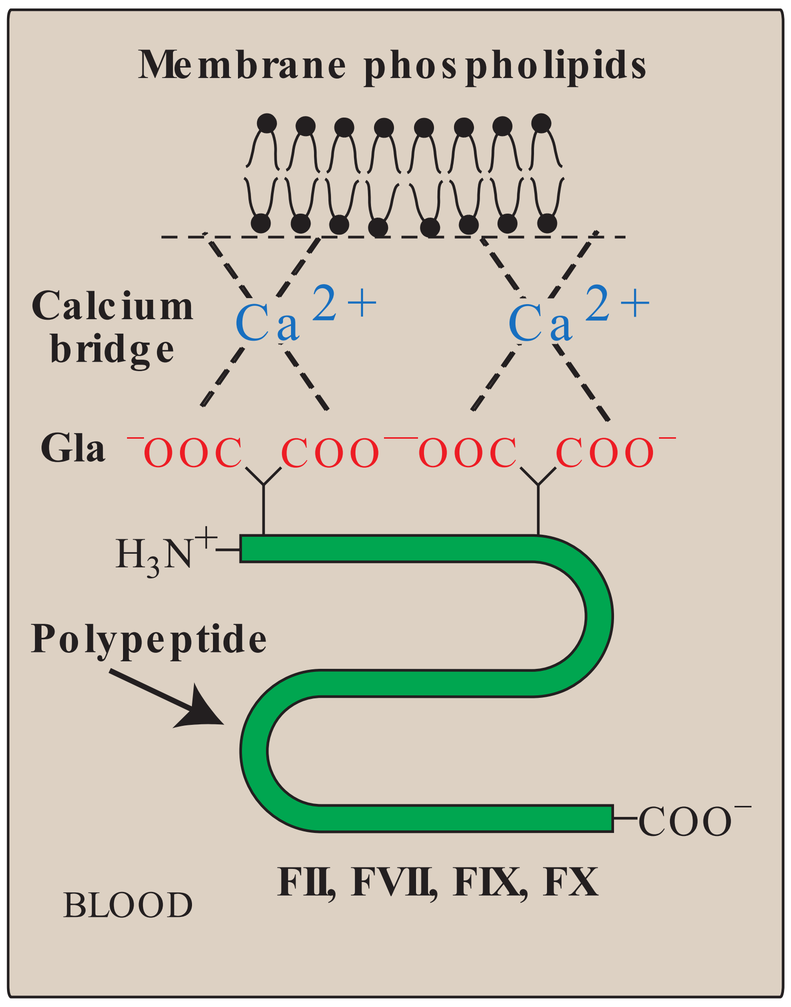


Figure 34.4
Ca^{2+} facilitates the binding of γ-carboxyglutamate (Gla)-containing factors to membrane phospholipids. F = factor.

C. Formation of g-carboxyglutamate residues

g-Carboxylation is a posttranslational modification in which 9–12 glutamate residues (at the amino or N terminus of the target protein) get carboxylated at the g carbon, thereby forming g-carboxyglutamate (Gla) residues. The process occurs in the rough endoplasmic reticulum (RER) of the liver.

1. **g-Carboxylation:** This carboxylation reaction requires a protein substrate, O_2, CO_2, g-glutamyl carboxylase, and the hydroquinone form of vitamin K as a coenzyme (Figure 34.6). In the reaction, the hydroquinone form of vitamin K gets oxidized to its epoxide form as O_2 is reduced to water. [Note: Vitamin K, a fat-soluble vitamin (see p. 389), is reduced from the quinone form to the hydroquinone coenzyme form by vitamin K reductase (Figure 34.7).]

2. **Inhibition by warfarin:** The formation of Gla residues is sensitive to inhibition by warfarin, a synthetic analog of vitamin K that inhibits the enzyme vitamin K epoxide reductase (VKOR). The reductase, an integral protein complex of the RER membrane, is required to regenerate the functional hydroquinone form of vitamin K from the epoxide form generated in the g-carboxylation reaction. Thus, warfarin is an anticoagulant that inhibits clotting by functioning as a vitamin K antagonist. Warfarin salts are used therapeutically to limit clot formation. [Note: Warfarin is used

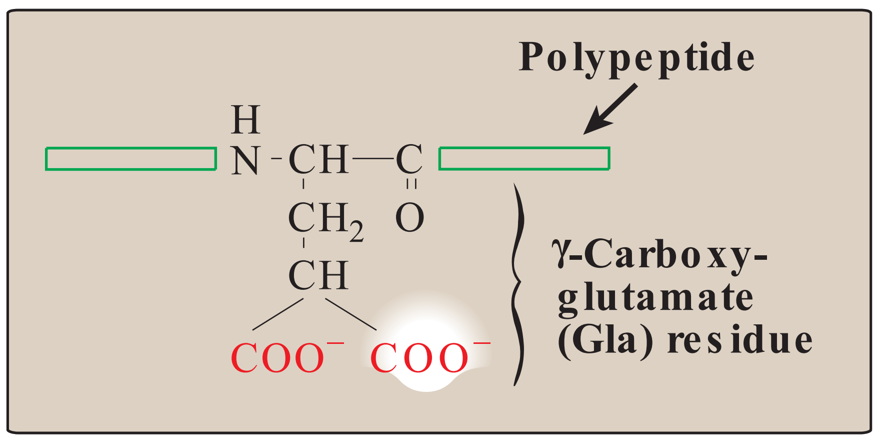


Figure 34.5
Gla residue.

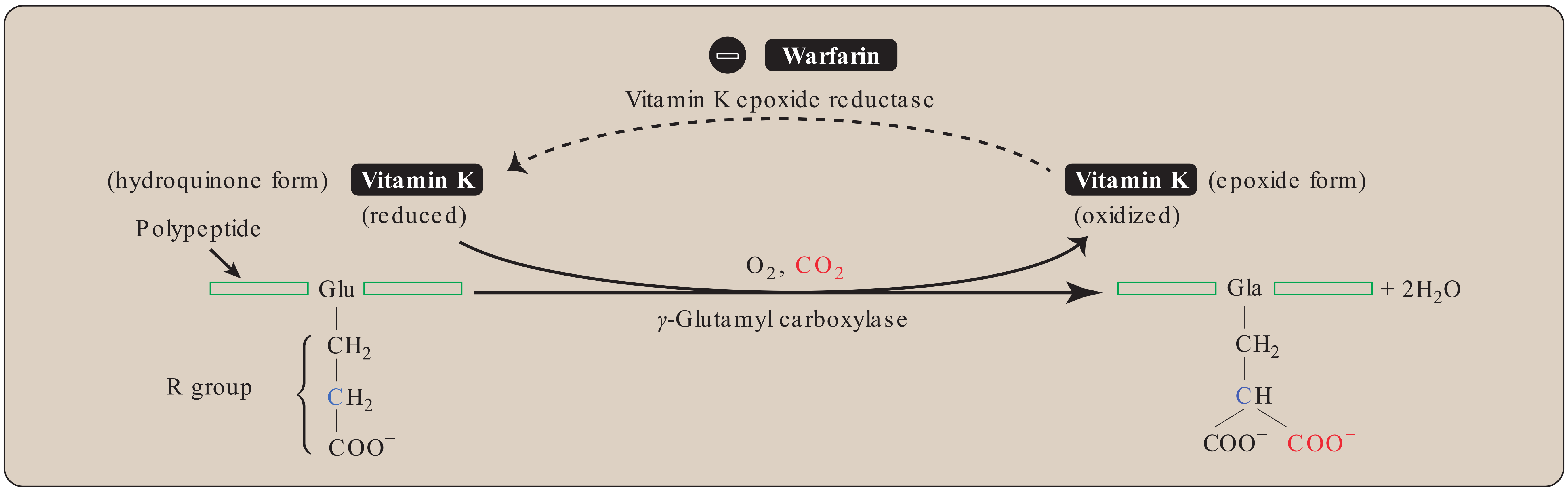

Warfarin
Vitamin K epoxide reductase
(hydroquinone form)
Vitamin K
(reduced)
Vitamin K
(epoxide form)
(oxidized)
Polypeptide
Glu
O_2, CO_2
γ-Glutamyl carboxylase
Gla
+ $2H_2O$
R group
CH_2
CH_2
COO^-
CH_2
CH
COO^-
COO^-

Figure 34.6
γ–Carboxylation of a glutamate (Glu) residue to γ-carboxyglutamate (Gla) by vitamin K–requiring γ–glutamyl carboxylase. The γ carbon is shown in blue.

commercially as a pest-control agent such as in rat poison. It was developed by the Wisconsin Alumni Research Foundation, hence the name.]

> Genetic differences (genotypes) in the gene for subunit 1 of the VKOR complex (VKORC1) influence patient response to warfarin. For example, a polymorphism in the promoter region of the gene decreases gene expression, resulting in less VKOR being made, thereby necessitating a lower dose of warfarin to achieve a therapeutic level. Polymorphisms in the cytochrome P450 enzyme (CYP2C9) that metabolizes warfarin are also known. In 2010, the U.S. Food and Drug Administration added a genotype-based dose table to the warfarin label (package insert). The influence of genetics on an individual's response to drugs is known as pharmacogenetics.

D. Pathways

Three distinct pathways are involved in formation of the fibrin meshwork: the extrinsic pathway, the intrinsic pathway, and the common pathway. Production of FXa by the extrinsic and intrinsic pathways initiates the common pathway (see Figure 34.2).

1. **Extrinsic pathway:** This pathway involves a protein, tissue factor (TF), that is not in the blood but becomes exposed when blood vessels get injured. TF (FIII) is a transmembrane glycoprotein abundant in vascular subendothelium. It is an extravascular accessory protein and not a protease. Any injury that exposes FIII to blood rapidly (within seconds) initiates the extrinsic (or TF) pathway. Once exposed, TF binds a circulating Gla-containing protein, FVII, activating it through conformational change. [Note:

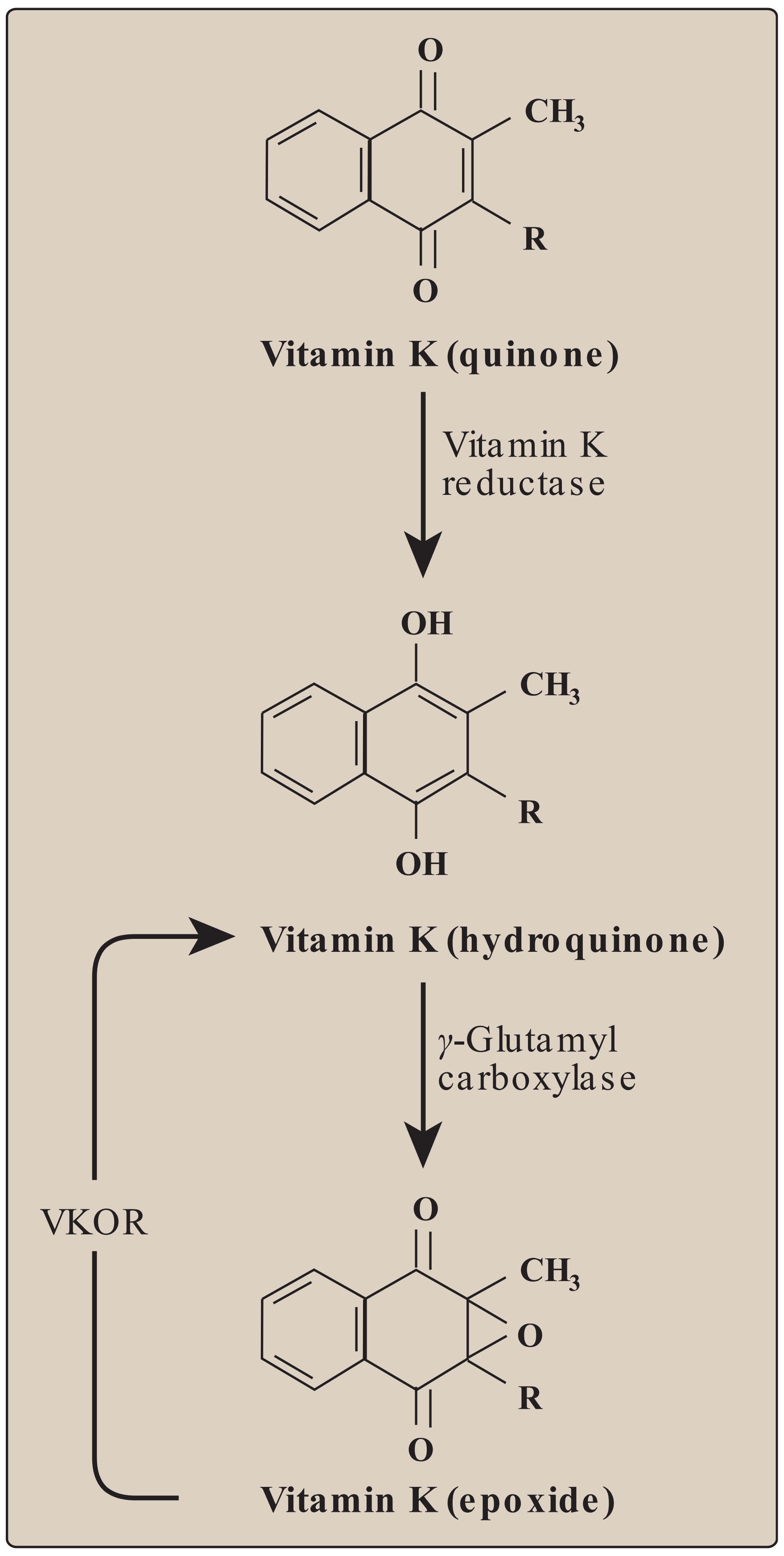

O
CH_3
R
O
Vitamin K (quinone)
Vitamin K reductase
OH
CH_3
R
OH
Vitamin K (hydroquinone)
γ-Glutamyl carboxylase
VKOR
O
CH_3
O
R
O
Vitamin K (epoxide)

Figure 34.7
The vitamin K cycle. VKOR = vitamin K epoxide reductase.

FVII can also be activated proteolytically by thrombin (see Section 3. below).] Binding of FVII to TF requires the presence of Ca^{2+} and phospholipids. The TF–FVIIa complex then binds and activates FX by proteolysis (Figure 34.8). Therefore, activation of FX by the extrinsic pathway occurs in association with the membrane. The extrinsic pathway is quickly inactivated by tissue factor pathway inhibitor (TFPI) that, in a FXa-dependent process, binds to the TF–FVIIa complex and prevents further production of FXa. [Note: TF and FVII are unique to the extrinsic pathway.]

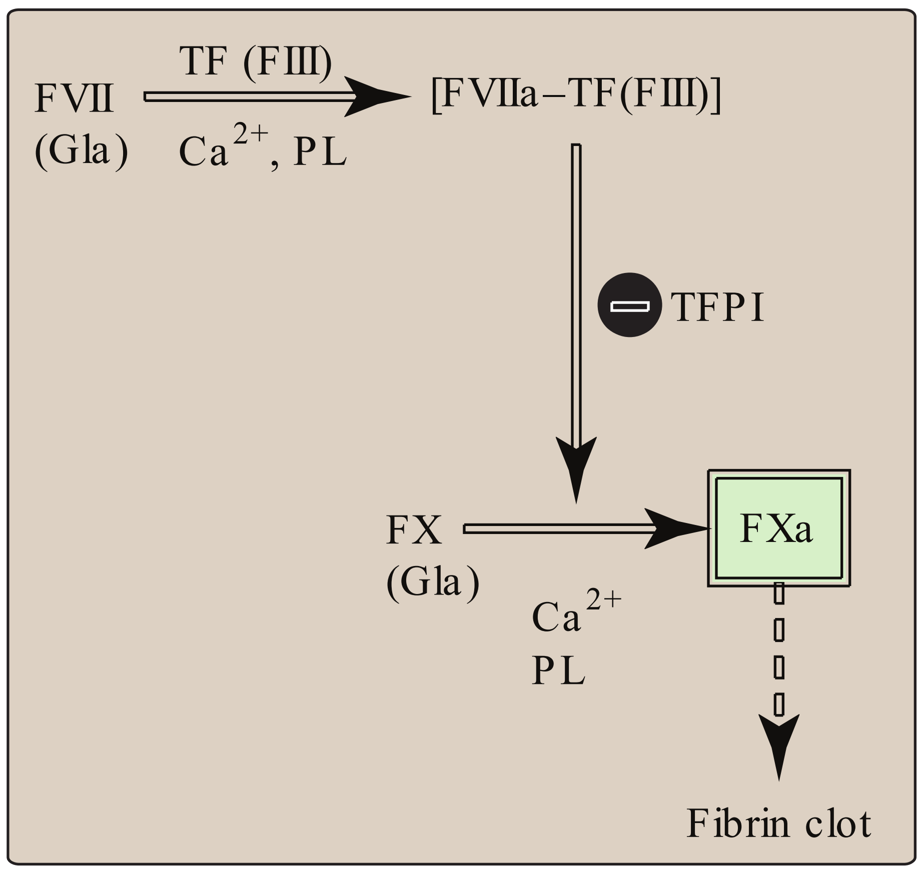


Figure 34.8
The extrinsic or tissue factor (TF) pathway. Binding of FVII to exposed TF (FIII) activates FVII. [Note: The pathway is quickly inhibited by tissue factor pathway inhibitor (TFPI).] F = factor; Gla = γ-carboxyglutamate; PL = phospholipid; a = active.

2. **Intrinsic pathway:** All of the protein factors involved in the intrinsic pathway are present in the blood and are, therefore, intravascular. The intrinsic pathway involves two phases: the contact phase and the FX-activation phase, each with known deficiencies.

   a. **Contact phase:** This phase results in the activation of FXII (Hageman factor) to FXIIa by conformational change through binding to a negative surface. Deficiencies in FXII (or in the other proteins of this phase, high-molecular-weight kininogen and prekallikrein) do not result in bleeding problems, calling into question the importance of this phase in coagulation. However, the contact phase does play a role in inflammation. [Note: FXII can be activated proteolytically by thrombin (see Section 3. below)].

   b. **Factor X–activation phase:** The sequence of events leading to the activation of Factor X to FXa by the intrinsic pathway is initiated by FXIIa (Figure 34.9). FXIIa activates FXI, and FXIa activates FIX, a Gla-containing protein. FIXa combines with FVIIIa (a bloodborne accessory [nonenzymatic] protein), and the complex activates FX, a Gla-containing serine protease. [Note: The complex containing FIXa, FVIIIa, and FX forms on exposed negatively charged membrane regions, and FX gets activated to FXa. This complex is sometimes referred to as Xase. Binding of the complex to membrane phospholipids requires Ca^{2+}.]

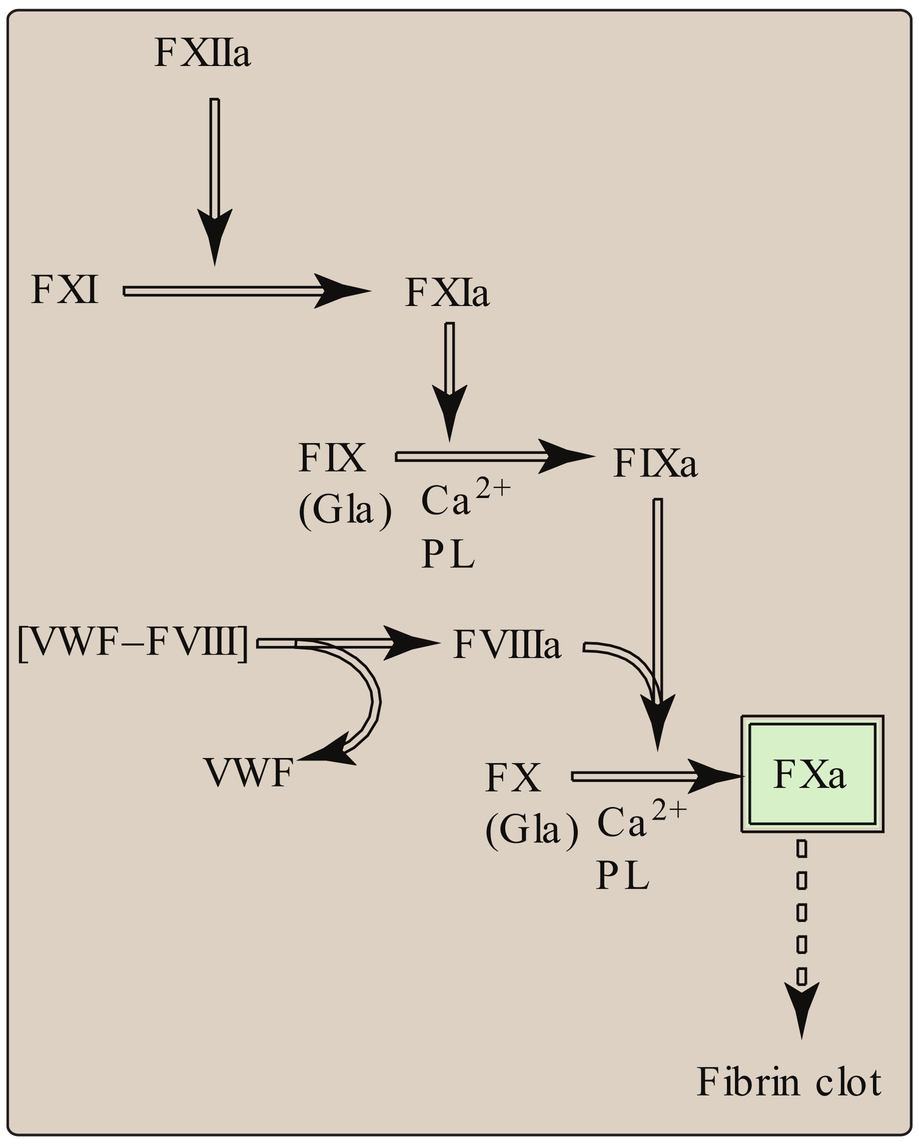


Figure 34.9
FX activation phase of the intrinsic pathway. [Note: von Willebrand factor (VWF) stabilizes FVIII in the circulation.] Gla = γ-carboxyglutamate; PL = phospholipid; a = active; F = factor.

   c. **Factor XII deficiency:** A deficiency in FXII does not lead to a bleeding disorder. This is because FXI, the next protein in the cascade, can be activated proteolytically by thrombin (see Section 3. below).

   d. **Hemophilia:** Hemophilia is a coagulopathy, a defect in the ability to clot. Hemophilia A (the most common form of hemophilia) results from deficiency of FVIII, whereas deficiency of FIX results in hemophilia B. Each deficiency is characterized by decreased and delayed ability to clot and/or formation of abnormally friable (easily disrupted) clots. This can be manifested, for example, by bleeding into the joints (Figure 34.10). The extent of the factor deficiency determines the severity of the disease. Current treatment for hemophilia is factor replacement therapy using factors obtained from pooled human blood or from recombinant DNA technology. Gene replacement therapy is a goal. Because the genes for both proteins are on the X chromosome, hemophilia is an X-linked disorder. [Note: Deficiency of FXI results in a bleeding disorder that sometimes is referred to as hemophilia C.]

> The inactivation of the extrinsic pathway by TFPI results in dependence on the intrinsic pathway for continued production of FXa. This explains why individuals with hemophilia bleed even though they have an intact extrinsic pathway.

3. **Common pathway:** FXa produced by both the intrinsic and the extrinsic paths initiates the common pathway, a sequence of events that results in the generation of fibrin (FIa) (Figure 34.11). FXa associates with FVa (a bloodborne accessory [nonenzymic] protein) and, in the presence of Ca^{2+} and phospholipids, forms a membrane-bound complex referred to as prothrombinase. The complex cleaves prothrombin (FII) to thrombin (FIIa). [Note: FVa potentiates the proteolytic activity of FXa.] The binding of Ca^{2+} to the Gla residues in FII facilitates the binding of FII to the membrane and to the prothrombinase complex, with subsequent cleavage to thrombin. Cleavage excises the Gla-containing region, releasing thrombin from the membrane and, thereby, freeing it to activate fibrinogen (FI) in the blood. [Note: This is the only example of cleavage of a Gla protein that results in the release of a Gla-containing peptide. The peptide travels to the liver where it is thought to act as a signal for increased production of clotting proteins.] Oral, direct inhibitors of FXa have been approved for limited clinical use as anticoagulants.

> A common point mutation (G20210A) in which an adenine (A) replaces a guanine (G) at nucleotide 20210 in the 3′ untranslated region of the gene for prothrombin leads to increased levels of prothrombin in the blood. This results in thrombophilia, a condition characterized by an increased tendency to clot.

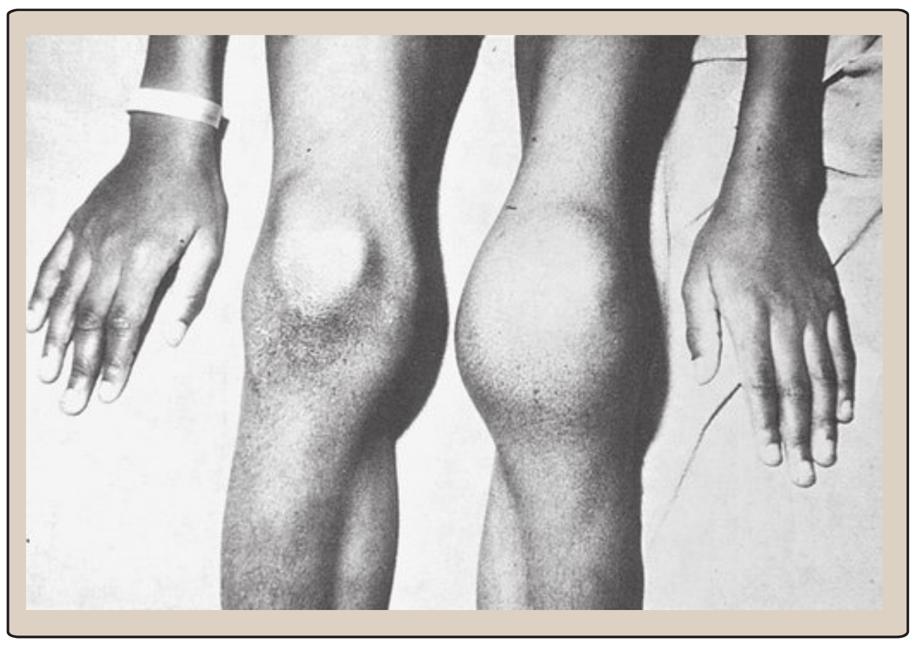


Figure 34.10
Acute bleeding into joint spaces (hemarthrosis) in an individual with hemophilia.

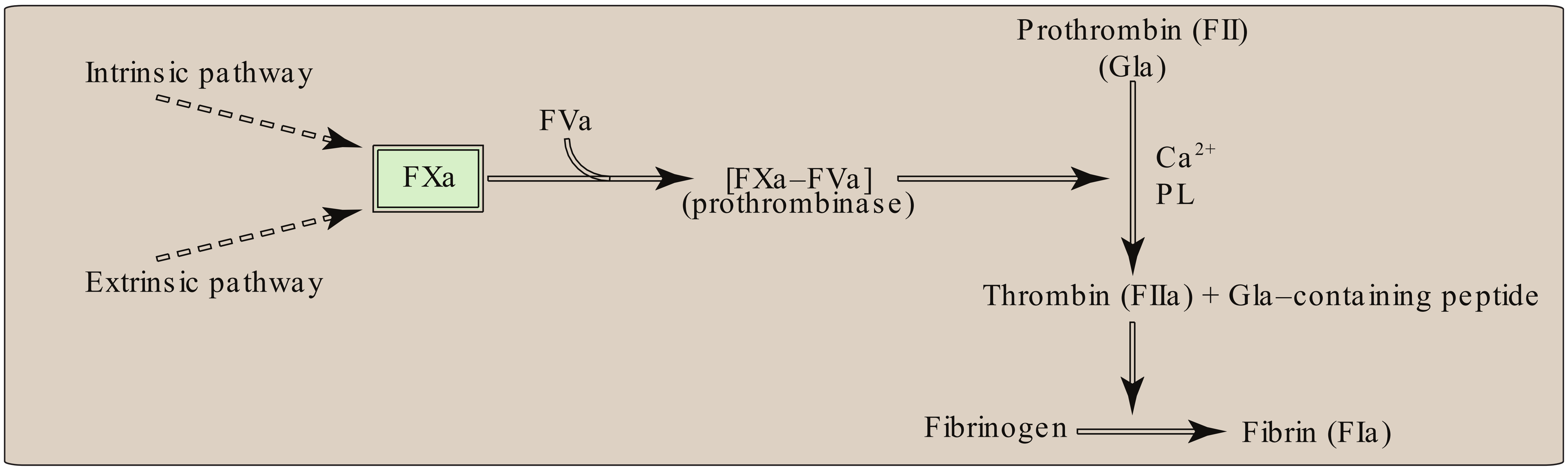


Figure 34.11
Generation of fibrin by FXa and the common pathway. F = factor; Gla = γ-carboxyglutamate; PL = phospholipid; a = active.

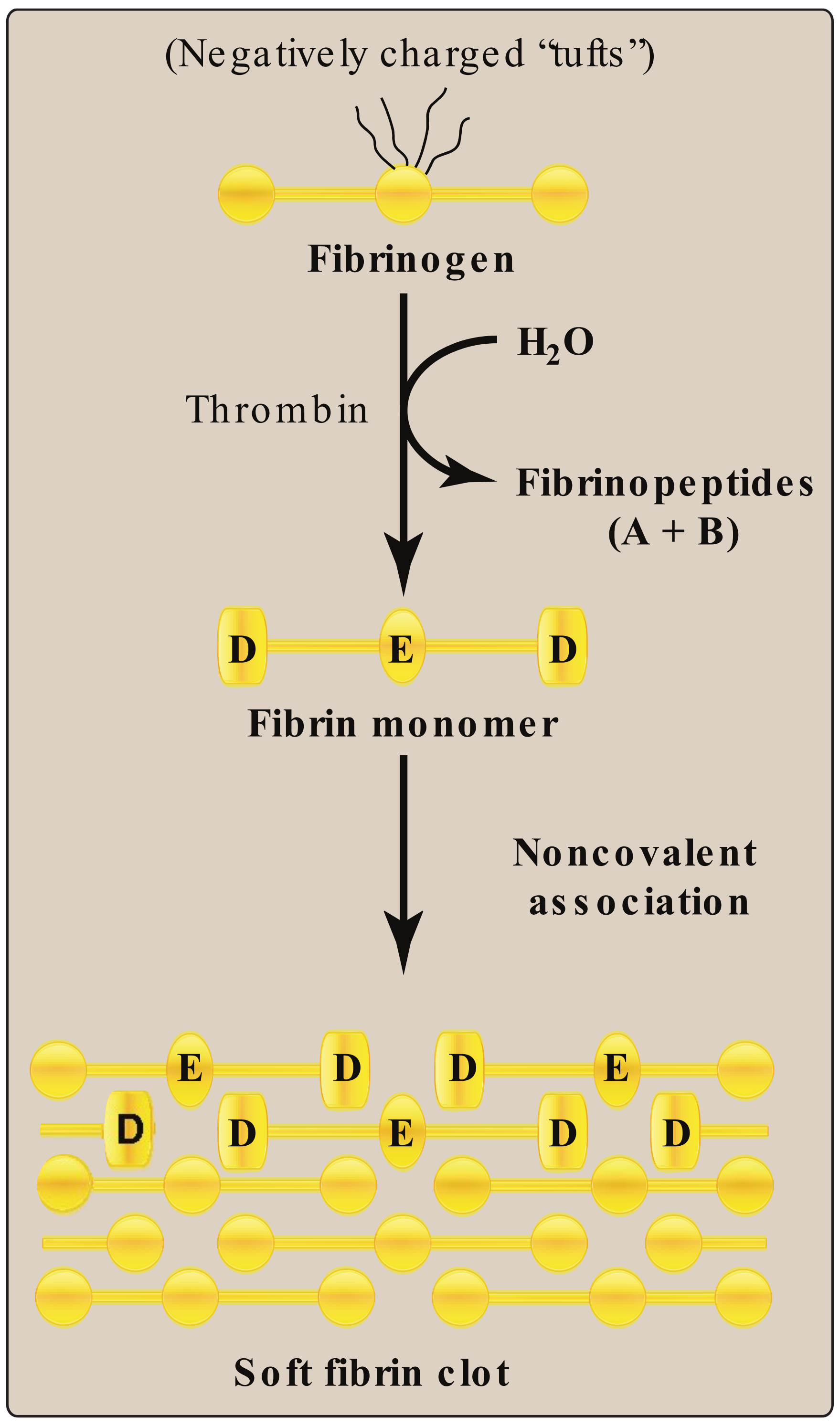

(Negatively charged "tufts")
Fibrinogen
Thrombin
H_2O
Fibrinopeptides (A + B)
D
E
D
Fibrin monomer
Noncovalent association
E
D
D
E
D
D
E
D
D
Soft fibrin clot

Figure 34.12
Conversion of fibrinogen to fibrin and formation of the soft fibrin clot. [Note: D and E refer to domains on fibrin.]

a. **Conversion of fibrinogen to fibrin by thrombin:** Fibrinogen is a soluble glycoprotein made by the liver. It consists of dimers of three different polypeptide chains $[(A\alpha)_2(B\beta)_2(g)_2]$ held together at the N termini by disulfide bonds. [Note: Aα and Bβ each represent a single polypeptide.] The N termini of the Aα and Bβ chains form "tufts" on the central of three globular domains (Figure 34.12). The tufts are negatively charged and result in repulsion between fibrinogen molecules. Thrombin cleaves the charged tufts (releasing fibrinopeptides A and B), and fibrinogen becomes fibrin. As a result of the loss of charge, the fibrin monomers are able to noncovalently associate in a staggered array, and a soft (soluble) fibrin clot is formed.

b. **Cross-linking of fibrin:** The associated fibrin molecules get covalently cross-linked. This converts the soft clot to a hard (insoluble) clot. FXIIIa, a transglutaminase, covalently links the g-carboxamide of a glutamine residue in one fibrin molecule to the ε-amino of a lysine residue in another through formation of an isopeptide bond and release of ammonia (Figure 34.13). [Note: FXIII is also activated by thrombin.]

c. **Importance of thrombin:** The activation of FX by the extrinsic path provides the "spark" of FXa that results in the initial activation of thrombin. Active thrombin then activates factors of the common (FV, FI, FXIII), intrinsic (FXI, FVIII), and extrinsic (FVII) pathways (Figure 34.14). It also activates FXII of the contact phase. The extrinsic pathway, then, initiates clotting by the generation of FXa, and the intrinsic pathway amplifies and sustains clotting after the extrinsic pathway has been inhibited by TFPI. [Note: Hirudin, a peptide secreted from the salivary gland of medicinal leeches, is a potent, direct, oral thrombin inhibitor. Recombinant hirudin has been approved for limited clinical use.] Additional crosstalk between the pathways of clotting is achieved by the FVIIa–TF-mediated activation of the intrinsic pathway and the FXIIa-mediated activation of the extrinsic pathway. The complete picture of physiologic blood clotting via the formation of a hard fibrin clot is shown in Figure 34.15. The factors of the clotting cascade are shown organized by function in Figure 34.16.

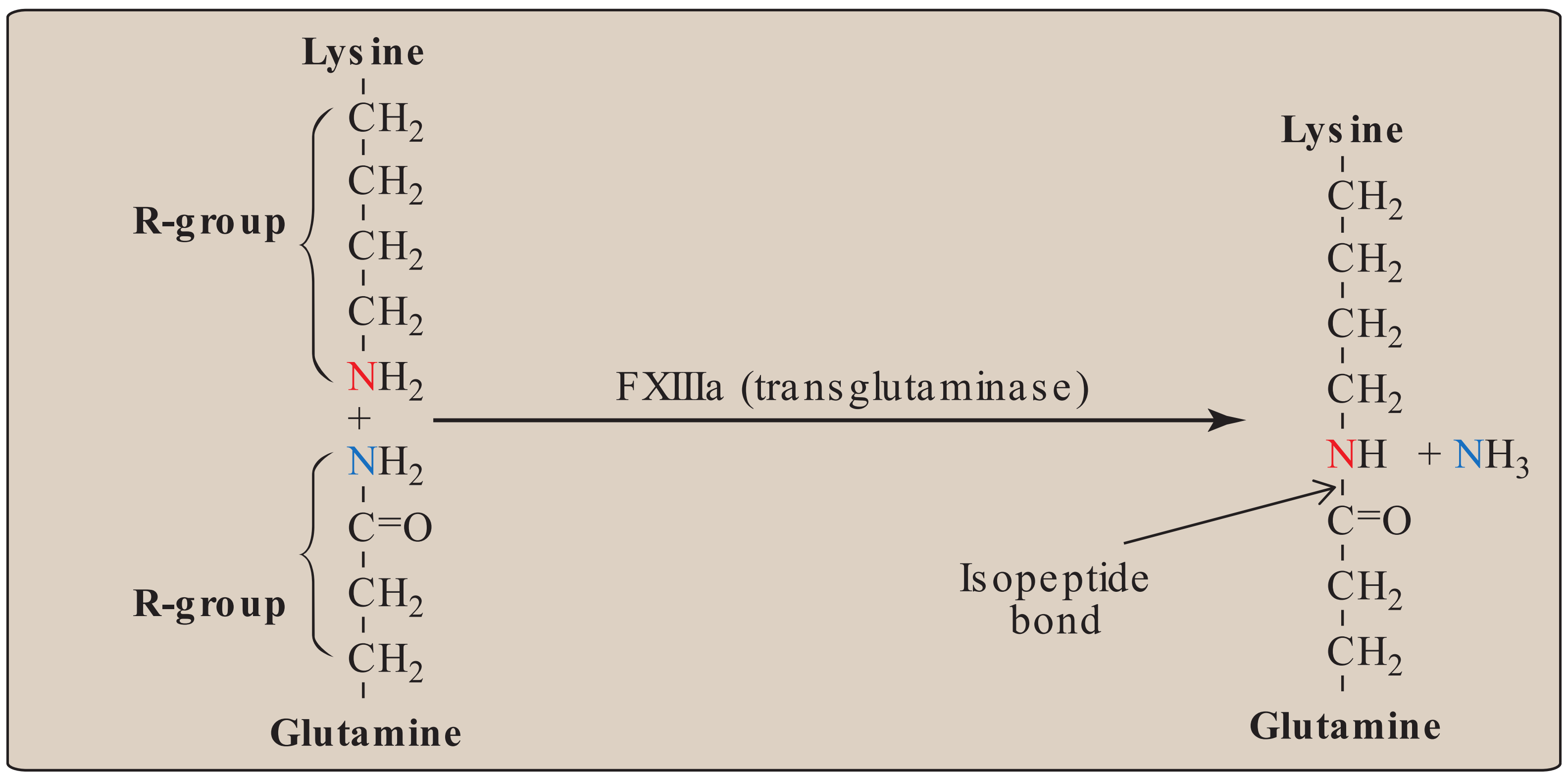

Lysine
CH_2
CH_2
CH_2
CH_2
R-group
NH_2
+
NH_2
$C{=}O$
CH_2
CH_2
R-group
Glutamine
FXIIIa (transglutaminase)
Lysine
CH_2
CH_2
CH_2
CH_2
NH $+ NH_3$
$C{=}O$
CH_2
CH_2
Isopeptide bond
Glutamine

Figure 34.13
Cross-linking of fibrin. FXIIIa forms a covalent isopeptide bond between a lysine residue and a glutamine residue. F = factor.

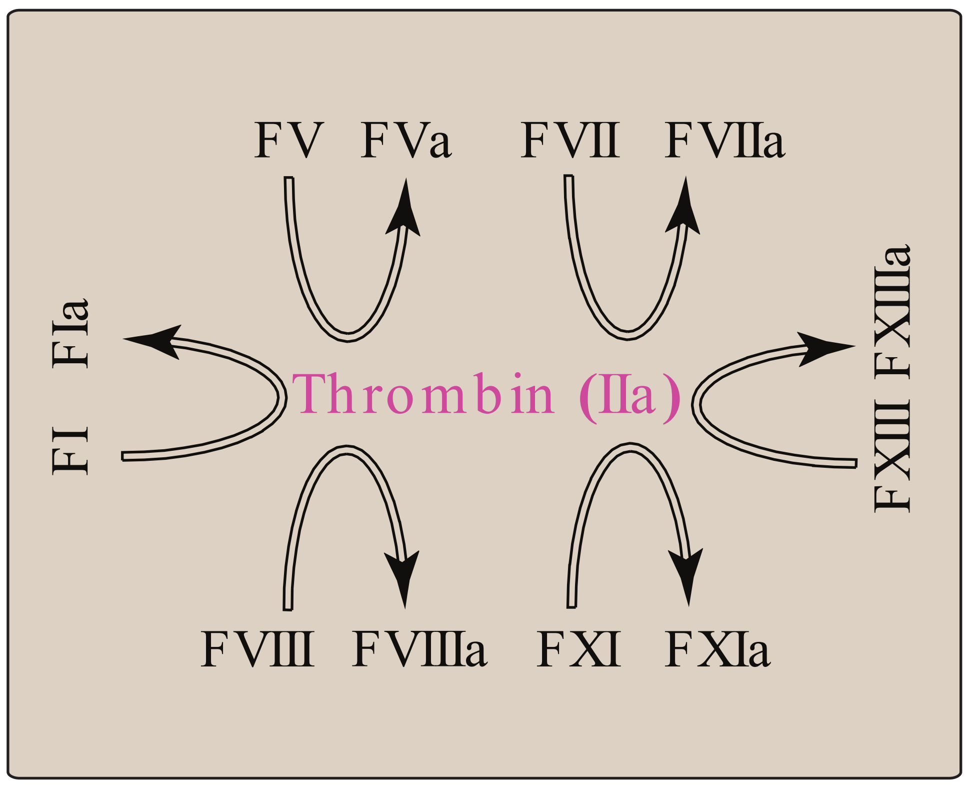

FV
FVa
FVII
FVIIa
FI
FIa
Thrombin (IIa)
FXIII
FXIIIa
FVIII
FVIIIa
FXI
FXIa

Figure 34.14
The importance of thrombin in formation of the fibrin clot. a = active; F = factor.

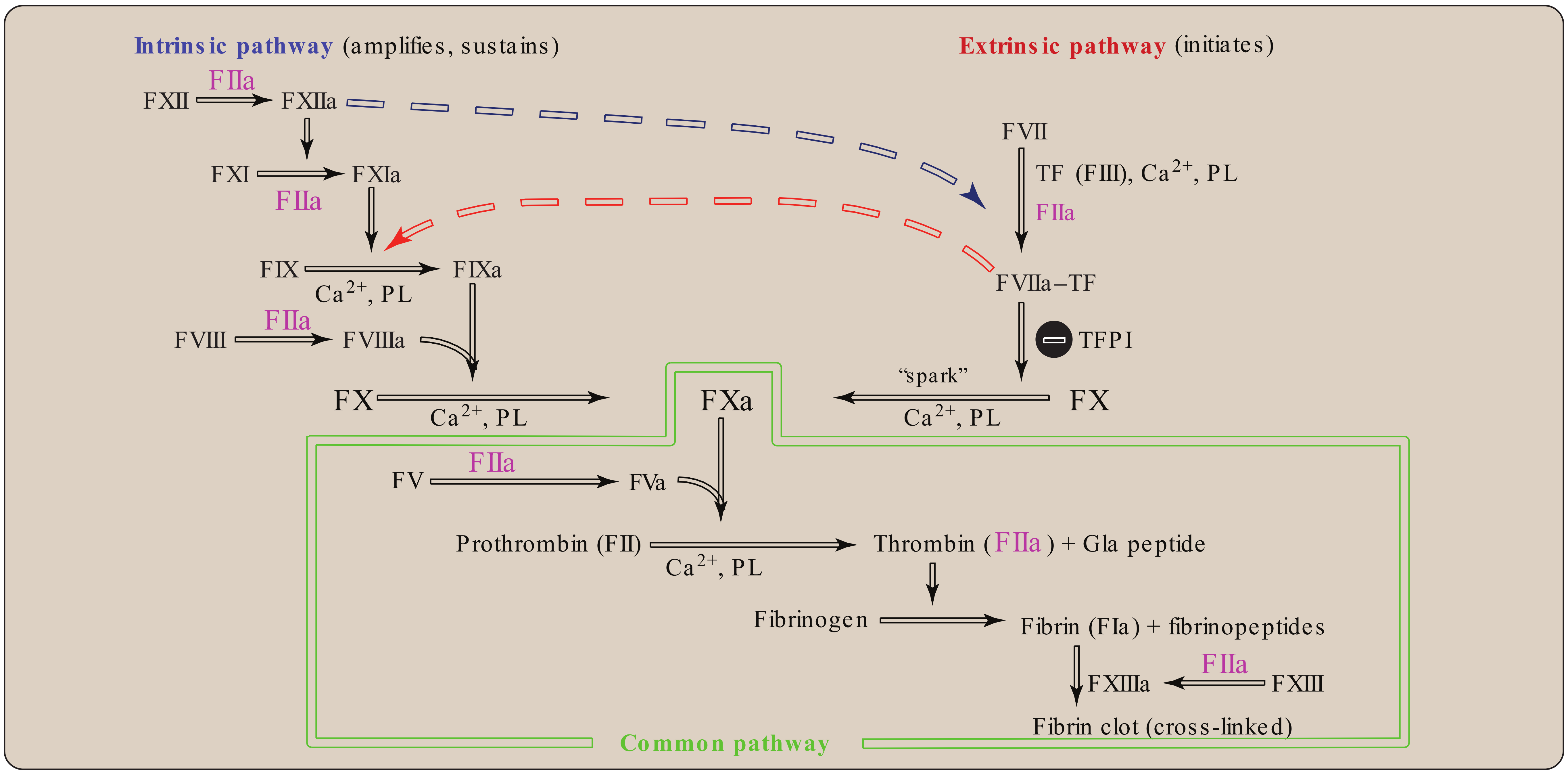

Intrinsic pathway (amplifies, sustains)
Extrinsic pathway (initiates)
FIIa
FXII
FXIIa
FVII
FXI
FXIa
TF (FIII), Ca2+, PL
FIIa
FIIa
FIX
FIXa
FVIIa–TF
Ca2+, PL
FIIa
FVIII
FVIIIa
TFPI
"spark"
FX
Ca2+, PL
FXa
FX
Ca2+, PL
FV
FIIa
FVa
Prothrombin (FII)
Thrombin (FIIa) + Gla peptide
Ca2+, PL
Fibrinogen
Fibrin (FIa) + fibrinopeptides
FIIa
FXIIIa
FXIII
Fibrin clot (cross-linked)
Common pathway

Figure 34.15
The complete picture of physiologic blood clotting via the formation of a cross-linked (hard) fibrin clot. a = active; F = factor; TF = tissue factor; TFPI = tissue factor pathway inhibitor; PL = phospholipid; Gla = γ-carboxyglutamate.

Clinical laboratory tests are available to evaluate the extrinsic through common pathways (prothrombin time [PT] using thromboplastin and expressed as the International Normalized Ratio [INR]) and the intrinsic through common pathways (activated partial thromboplastin time [aPTT]). Thromboplastin is a combination of phospholipids + FIII. A derivative, partial thromboplastin, contains just the phospholipid portion because FIII isn't needed to activate the intrinsic pathway.

Serine proteases	II, VII, IX, X, XI, XII
Gla-containing proteases	II, VII, IX, X
Accessory proteins	III, V, VIII

Figure 34.16
Protein factors of the clotting cascade organized by function. The activated form would be denoted by an a after the numeral. [Note: Ca^{2+} is IV. There is no VI. I (fibrin) is neither a protease nor an accesory protein. XIII is a
.] Gla = γ-carboxyglutamate.

III. LIMITING CLOTTING

The ability to limit clotting to areas of damage (anticoagulation) and to remove clots once repair processes are underway (fibrinolysis) are exceedingly important aspects of hemostasis. These actions are performed by proteins that inactivate clotting factors either by binding to them and removing them from the blood or by degrading them and by proteins that degrade the fibrin meshwork.

A. Inactivating proteins

Proteins synthesized by the liver and by the blood vessels themselves balance the need to form clots at sites of vessel injury with the need to limit their formation beyond the injured area.

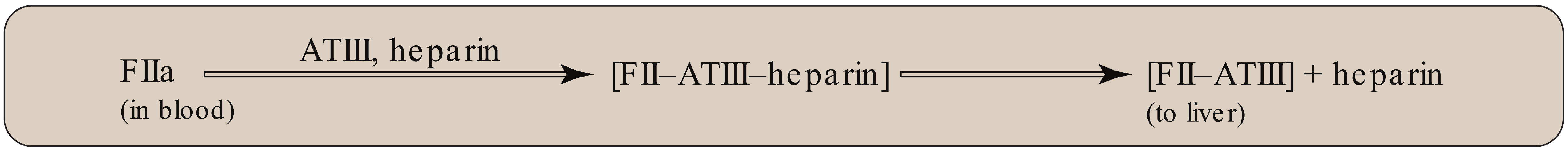


Figure 34.17
Inactivation of FIIa (thrombin) by binding of antithrombin III (ATIII) and transport to the liver. [Note: Heparin increases the affinity of ATIII for FIIa.] a = active; F = factor.

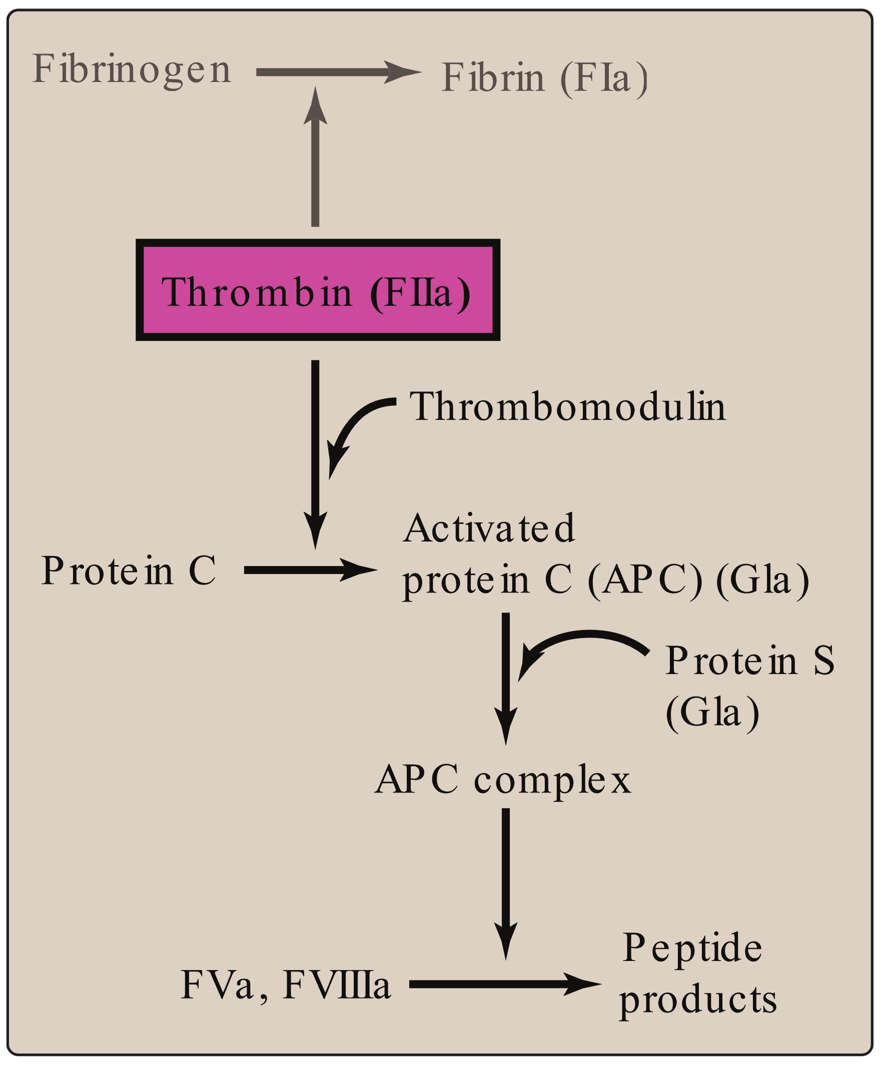


Figure 34.18
Formation and action of the APC complex. Gla = γ-carboxyglutamate; a = active; F = factor.

1. **Antithrombin:** Antithrombin III (ATIII), also referred to simply as antithrombin (AT), is a hepatic protein that circulates in the blood. It inactivates free thrombin by binding to it and carrying it to the liver (Figure 34.17). Thus, ATIII removes thrombin from the blood, preventing it from participating in coagulation. [Note: ATIII is a serine protease inhibitor, or "serpin." A serpin contains a reactive loop to which a specific protease binds. Once bound, the protease cleaves a peptide bond in the serpin causing a conformational change that traps the enzyme in a covalent complex. α_1-Antitrypsin (see p. 50) is also a serpin.] The affinity of ATIII for thrombin is greatly increased when ATIII is bound to heparin, an intracellular glycosaminoglycan released in response to injury by mast cells associated with blood vessels. Heparin, an anticoagulant, is used therapeutically to limit clot formation. [Note: In contrast to the anticoagulant warfarin, which has a slow onset, a long half-life, and is administered orally, heparin has a rapid onset, a short half-life, and requires intravenous administration. The two drugs are commonly used in an overlapping manner in the treatment of thrombosis.] ATIII also inactivates FXa and the other serine proteases of clotting, FIXa, FXIa, FXIIa, and the FVIIa–TF complex. [Note: AT binds to a specific pentasaccharide within the oligosaccharide form of heparin. Inhibition of FIIa requires the oligosaccharide form, whereas inhibition of FXa requires only the pentasaccharide form. Fondaparinux, a synthetic version of the pentasaccharide, is used clinically to inhibit FXa.]

2. **Protein C–protein S complex:** Protein C, a circulating Gla-containing protein made in the liver, is activated by thrombin complexed with thrombomodulin. Thrombomodulin, an integral membrane glycoprotein of endothelial cells, binds thrombin, thereby decreasing thrombin's affinity for fibrinogen and increasing its affinity for protein C. Protein C in complex with protein S, also a Gla-containing protein, forms the activated protein C (APC) complex that cleaves the accessory proteins FVa and FVIIIa that are required for maximal activity of FXa (Figure 34.18). Protein S helps anchor APC to the clot. Thrombomodulin, then, modulates the activity of thrombin, converting it from a protein of coagulation to a protein of anticoagulation, thereby limiting the extent of clotting. Factor V Leiden is a mutant form of FV (glutamine is substituted for arginine at position 506) that is resistant to APC. It is the most common inherited cause of thrombophilia in the United States, with highest frequency in the Caucasian population. Heterozygotes have a 7-fold increase in the risk for venous thrombosis, and homozygotes have up to a 50-fold increase. [Note: Women with FV Leiden are at even greater risk of thrombosis during pregnancy or when taking estrogen.]

Thrombophilia (hypercoagulability) can result from deficiencies of proteins C, S, and ATIII; from the presence of FV Leiden and antiphospholipid antibodies; and from excess production of prothrombin (G20210A mutation). [Note: A thrombus that forms in the deep veins of the leg (deep venous thrombosis, or DVT) can cause a pulmonary embolism (PE) if the clot (or a piece of it) breaks off, travels to the lungs, and blocks circulation.]

B. Fibrinolysis

Clots are temporary patches that must be removed once wound repair has begun. The fibrin clot is cleaved by the protein plasmin to fibrin-degradation products (Figure 34.19). [Note: Measurement of D-dimer, a fibrin-degradation product containing two cross-linked D domains released by the action of plasmin, can be used to assess the extent of clotting (see Figure 34.12).] Plasmin is a serine protease that is generated from plasminogen by plasminogen activators. Plasminogen, secreted by the liver into the circulation, binds to fibrin and is incorporated into clots as they form. Tissue plasminogen activator (TPA, t-PA), made by vascular endothelial cells and secreted in an inactive form in response to thrombin, becomes active when bound to fibrin–plasminogen. Bound plasmin and TPA are protected from their inhibitors, α_2-antiplasmin (a serpin) and plasminogen activator inhibitors, respectively. Once the fibrin clot is dissolved, plasmin and TPA become available to their inhibitors. Therapeutic fibrinolysis in patients with an MI or an ischemic stroke can be achieved by treatment with commercially available TPA made by recombinant DNA techniques. [Note: Urokinase is a plasminogen activator (u-PA) made in a variety of tissues and originally isolated from urine. Streptokinase (from bacteria) also activates plasminogen and works on both free and fibrin-bound plasminogen.]

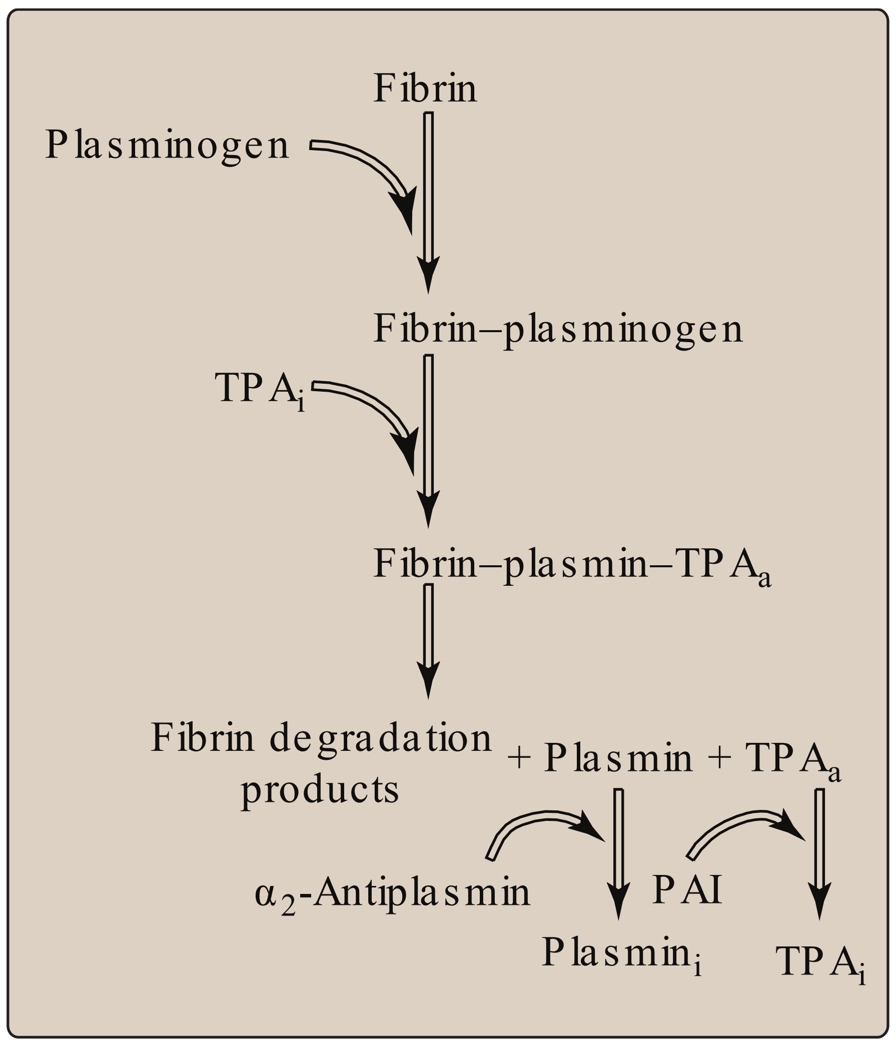


Figure 34.19
Fibrinolysis. TPA = tissue plasminogen activator; i = inactive; a = active; PAI = plasminogen activator inhibitor. [Note: Plasmin bound to fibrin is protected from its inhibitor.]

Plasminogen contains structural motifs known as kringle domains that mediate protein–protein interactions. Lipoprotein(a) [Lp(a)] also contains kringle domains and, thus, competes with plasminogen for binding to fibrin. The potential to inhibit fibrinolysis may be the basis for the association of elevated Lp(a) with increased risk for cardiovascular disease (see p. 236).

IV. PLATELET PLUG FORMATION

Platelets (thrombocytes) are small, anucleate fragments of megakaryocytes that adhere to exposed collagen of damaged endothelium, get activated, and aggregate to form a platelet plug (Figure 34.20). Formation of the platelet plug is referred to as primary hemostasis. In a

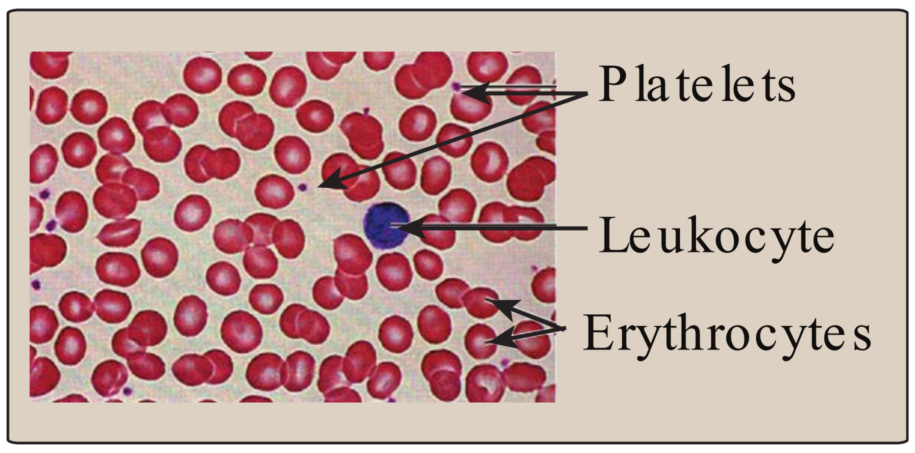


Figure 34.20
Size comparison of platelets, erythrocytes, and a leukocyte.

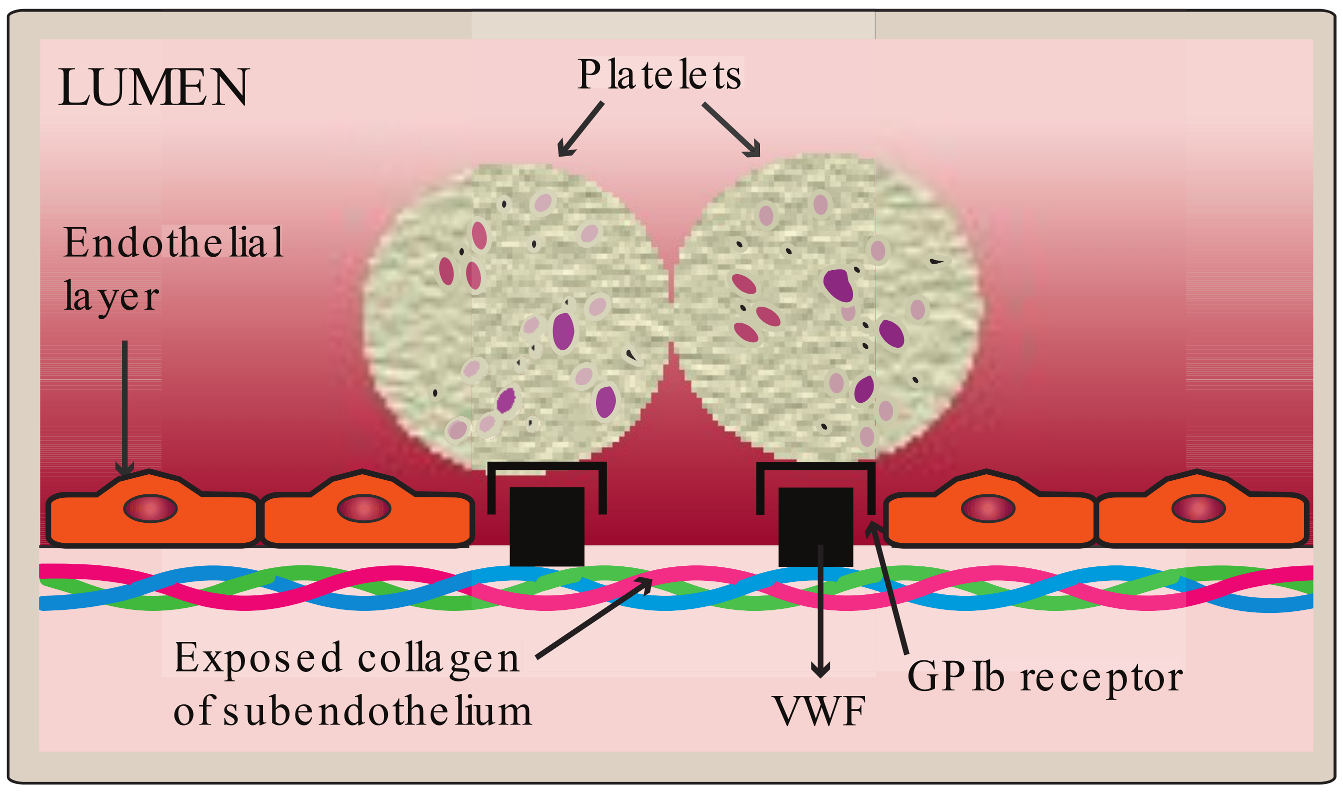

LUMEN
Platelets
Endothelial layer
Exposed collagen of subendothelium
VWF
GPIb receptor

Figure 34.21
Binding of platelets via the receptor glycoprotein Ib (GPIb) to von Willebrand factor (VWF). VWF is bound to the exposed collagen at a site of injury.

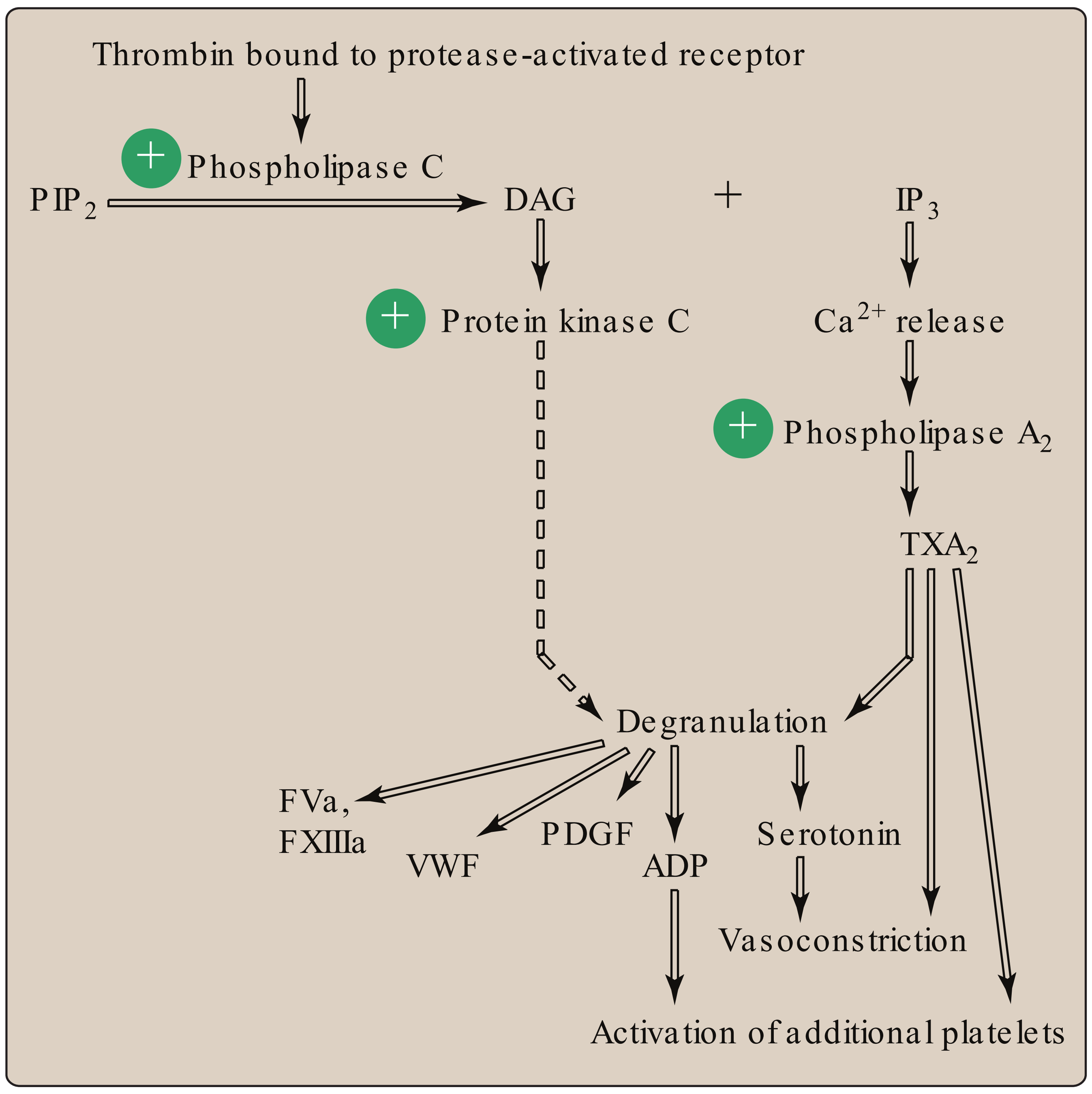

Thrombin bound to protease-activated receptor
+ Phospholipase C
PIP$_2$
DAG
+
IP$_3$
+ Protein kinase C
Ca^{2+} release
+ Phospholipase A$_2$
TXA$_2$
Degranulation
FVa, FXIIIa
VWF
PDGF
ADP
Serotonin
Vasoconstriction
Activation of additional platelets

Figure 34.22
Platelet activation by thrombin. [Note: Protease-activated receptors are a type of G protein–coupled receptor.] PIP_2 = phosphoinositol bisphosphate; DAG = diacylglycerol; IP_3 = inositol trisphosphate; TXA_2 = thromboxane A_2; ADP = adenosine diphosphate; PDGF = platelet-derived growth factor; VWF = von Willebrand factor; F = factor.

normal adult there are 150,000–450,000 platelets per μl of blood. They have a lifespan of up to 10 days, after which they are taken up by the liver and spleen and destroyed. Clinical laboratory tests to measure platelet number and activity are available.

A. Adhesion

Adhesion of platelets to exposed collagen at the site of vessel injury is mediated by the protein von Willebrand factor (VWF). VWF binds to collagen, and platelets bind to VWF via glycoprotein Ib (GPIb), a component of a membrane receptor complex (GPIb–V–IX) on the platelet surface (Figure 34.21). Binding to VWF stops the forward movement of platelets. [Note: Deficiency in the receptor for VWF results in Bernard-Soulier syndrome, a disorder of decreased platelet function and number.] VWF is a glycoprotein that is released from platelets. It also is made and secreted by endothelial cells. In addition to mediating the binding of platelets to collagen, VWF also binds to and stabilizes FVIII in the blood. Deficiency of VWF results in von Willebrand disease (VWD), the most common inherited defect in the ability to clot (coagulopathy). VWD coagulopathy results from decreased binding of platelets to collagen and a deficiency in FVIII (due to increased degradation). Platelets can also bind directly to collagen via the membrane receptor GPVI. Once adhered, platelets get activated. [Note: Damage to the endothelium also exposes FIII, initiating the extrinsic pathway of blood clotting and activation of FX (see Figure 34.8).]

B. Activation

Once adhered to areas of injury, platelets get activated. Platelet activation involves morphologic (shape) changes and degranulation, the process by which platelets secrete the contents of their α and δ (or dense) storage granules. Activated platelets also expose PS on their surface. Thrombin is the most potent platelet activator. Thrombin binds to and activates protease-activated receptors, a type of G protein–coupled receptor (GPCR), on the surface of platelets (Figure 34.22). Thrombin is primarily associated with G_q proteins (see p. 205), resulting in activation of phospholipase C and a rise in diacylglycerol (DAG) and inositol trisphosphate (IP_3). [Note: Thrombomodulin, through its binding of thrombin, decreases the availability of thrombin for platelet activation (see Figure 34.18).]

1. **Degranulation**: DAG activates protein kinase C, a key event for degranulation. IP_3 causes the release of Ca^{2+} (from dense granules). The Ca^{2+} activates phospholipase A_2, which cleaves mem-

brane phospholipids to release arachidonic acid, the substrate for the synthesis of thromboxane A_2 (TXA_2) in activated platelets by cyclooxygenase-1 (COX-1) (see p. 214). TXA_2 causes vasoconstriction, augments degranulation, and binds to platelet GPCRs, causing activation of additional platelets. Recall that aspirin irreversibly inhibits COX and, consequently, TXA_2 synthesis and is referred to as an "antiplatelet" drug. Degranulation also results in release of serotonin and adenosine diphosphate (ADP) from dense granules. Serotonin causes vasoconstriction. ADP binds to GPCRs on the surface of platelets, activating additional platelets. [Note: Some antiplatelet drugs, such as clopidogrel, are ADP-receptor antagonists.] Platelet-derived growth factor (involved in wound healing), VWF, FV, FXIII, and fibrinogen are among other proteins released from α granules. [Note: Platelet-activating factor (PAF), an ether phospholipid (see p. 202) synthesized by a variety of cell types including endothelial cells and platelets, binds PAF receptors (GPCRs) on the surface of platelets and activates them.]

2. **Shape change:** The change in shape of activated platelets from discoidal to spherical with pseudopod-like processes that facilitate platelet–platelet and platelet–surface interactions (Figure 34.23) is initiated by the release of Ca^{2+} from dense granules. Ca^{2+} bound to calmodulin mediates the activation of myosin light-chain kinase that phosphorylates the myosin light chain (see p. 151), resulting in a major reorganization of the platelet cytoskeleton.

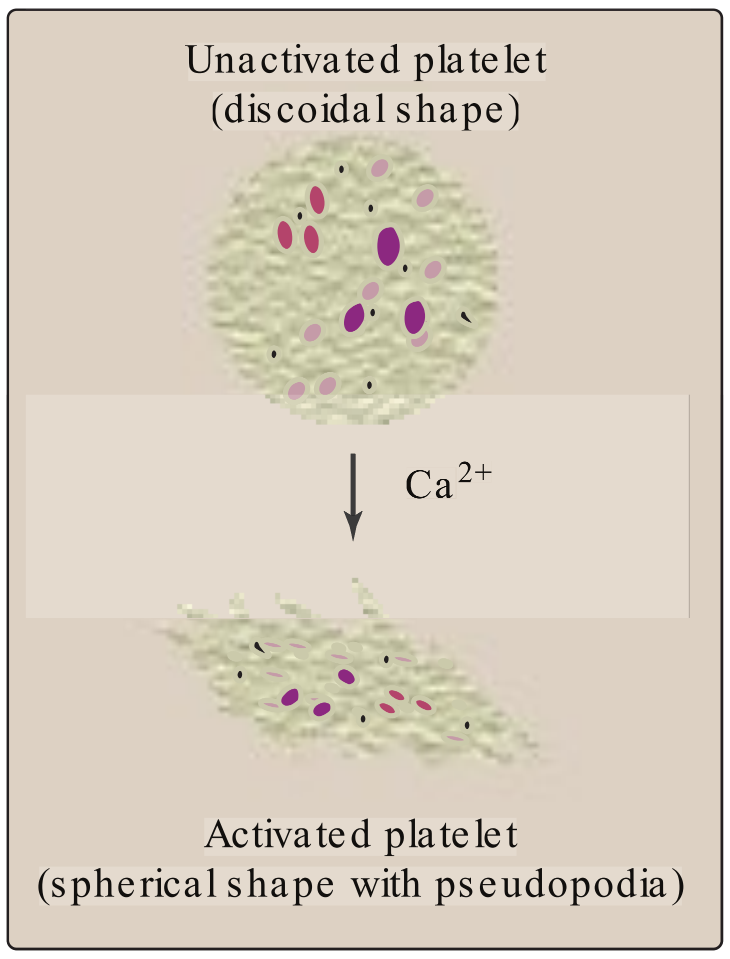


Figure 34.23
Activated platelets undergo Ca^{2+}-initiated shape change.

C. Aggregation

Activation causes dramatic changes in platelets that lead to their aggregation. Structural changes in a surface receptor (GPIIb/IIIa) expose binding sites for fibrinogen. Bound fibrinogen molecules link activated platelets to one another (Figure 34.24), with a single fibrinogen able to bind two platelets. The fibrinogen is converted to fibrin by thrombin and then covalently cross-linked by FXIIIa coming from both the blood and the platelets. [Note: The exposure of PS on the surface of activated platelets allows formation of the Xase complex (VIIIa, IXa, X, and Ca^{2+}) with subsequent formation of FXa and generation of thrombin.] Fibrin strengthens the platelet plug. [Note: Rare defects in the platelet receptor for fibrinogen result in Glanzmann thrombasthenia (decreased platelet function), whereas autoantibodies to this receptor are a cause of immune thrombocytopenia (decreased platelet number).]

> Unnecessary activation of platelets is prevented because 1) an intact vascular wall is separated from the blood by a monolayer of endothelial cells, preventing the contact of platelets with collagen; 2) endothelial cells synthesize prostaglandin I_2 (PGI_2, or prostacyclin) and nitric oxide, each of which causes vasodilation; and 3) endothelial cells have a cell-surface ADPase that converts ADP to AMP.

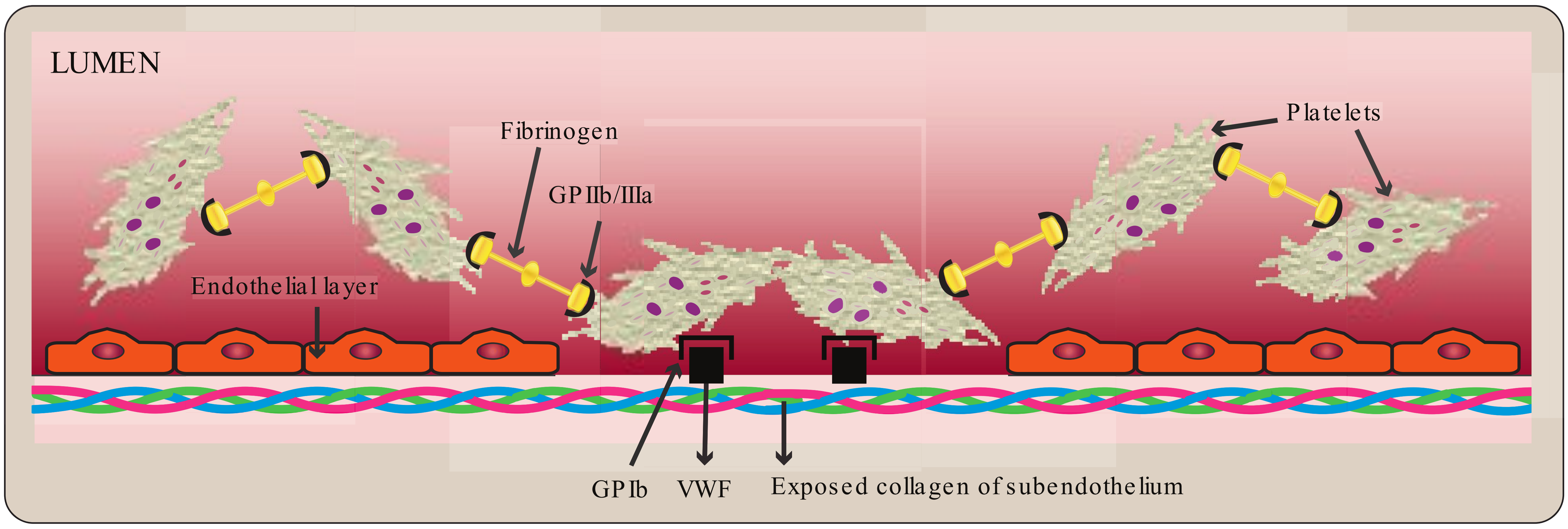

LUMEN
Fibrinogen
GP IIb/IIIa
Platelets
Endothelial layer
GP Ib
VWF
Exposed collagen of subendothelium

Figure 34.24
Linking of platelets by fibrinogen via the receptor glycoprotein (GP) IIb/IIIa. [Note: The shapes in the fibrinogen molecule represent the two D and one E domains.] GP Ib = glycoprotein Ib receptor; VWF = von Willebrand factor.

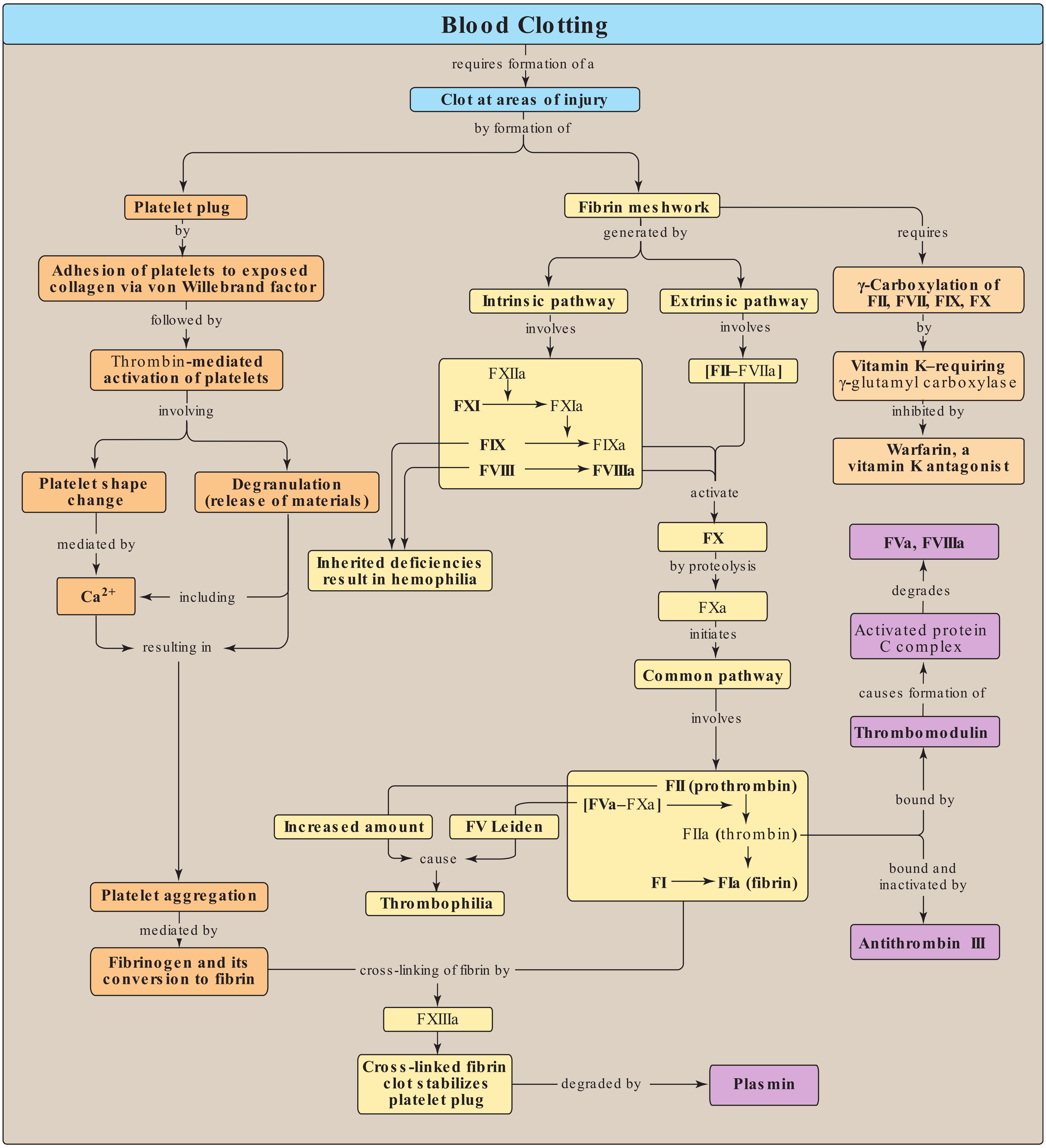

Blood Clotting
requires formation of a
Clot at areas of injury
by formation of
Platelet plug
by
Adhesion of platelets to exposed collagen via von Willebrand factor
followed by
Thrombin-mediated activation of platelets
involving
Platelet shape change
Degranulation (release of materials)
mediated by
Ca^{2+}
including
resulting in
Platelet aggregation
mediated by
Fibrinogen and its conversion to fibrin
Fibrin meshwork
generated by
Intrinsic pathway
involves
FXIIa
FXI
FXIa
FIX
FIXa
FVIII
FVIIIa
Extrinsic pathway
involves
[FIII–FVIIa]
activate
FX
by proteolysis
FXa
initiates
Common pathway
involves
FII (prothrombin)
[FVa–FXa]
FIIa (thrombin)
FI
FIa (fibrin)
Inherited deficiencies result in hemophilia
Increased amount
FV Leiden
cause
Thrombophilia
cross-linking of fibrin by
FXIIIa
Cross-linked fibrin clot stabilizes platelet plug
degraded by
Plasmin
requires
γ-Carboxylation of FII, FVII, FIX, FX
by
Vitamin K–requiring γ-glutamyl carboxylase
inhibited by
Warfarin, a vitamin K antagonist
FVa, FVIIIa
degrades
Activated protein C complex
causes formation of
Thrombomodulin
bound by
bound and inactivated by
Antithrombin III

Figure 34.25
Key concept map for blood clotting. a = active; F = factor.